THE HISTORY OF THE CHROMOSOMAL VESICLES IN FUNDULUS AND THE THEORY OF GENETIC CONTINUITY OF CHROMOSOMES.

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At the Columbus meeting of the American Society of Zoölogists in 1915, the writer reported the occurrence of chromosomal vesicles which could be recognized as persistent entities even in the resting stage. Since that time it has been possible to trace the history of these vesicles from the metaphase condition of the chromosomes through the anaphase, telophases and the various stages of interkinesis back to the prophase stage where the new chromosomes are fully formed as new bodies which are as distinctly chromosomal in character as the more familiar ones of the metaphase. This history can now be given with but few gaps; and these are of comparatively little importance. The bearing of these facts on the hypothesis of genetic continuity of the chromosomes is direct and very important.

The presence of persistent chromosomal vesicles was first noted in the study of the eggs of *Fundulus heteroclitus* which had been fertilized with the sperm of *Ctenolabrus* (*Tautogolabrus*) *adspersus*, and the history was first made out in a general way upon those cleavage nuclei. Subsequently the observations were extended to the *Fundulus* eggs fertilized normally by the sperm of the same species, and the gaps in the series filled in from this form. So far as could be determined there is no essential morphological distinction in the figures presented by the normal and the hybrid nuclei. The same account applies to both, with the exception of certain points to be noted later, which do not apply to the general facts and line of argument here given.

The first observations were made while working on the hybrid eggs to determine the effect of radiation upon the development, particularly with the reference to the chromosomes which differ in form and which can be recognized as distinct in the hybrid eggs as was shown by Miss Morris following the lines of the classical work of Moenkhaus.

In 1904 Moenkhaus's well-known paper appeared announcing the independence of two chromosome groups on the spindles of dividing eggs of *Fundulus* and *Menidia* hybrids, and the recognition, based on morphological evidence, of the two groups as from the male and female parents respectively. Conklin (1901) had already recognized the male and female halves of *Crepidula* cleavage nuclei; Ruckert, (1895) and Häcker (1895, 1902) had seen the double nuclei and bilateral distribution of chromatin in *Cyclops;* and Herla (1893) and Zoja (1895) had traced the independent maternal and paternal chromosomes of *Ascaris* eggs to the twelve-cell stage. Moenkhaus, after identifying in the normal eggs the chromosomes of *Fundulus* as straight rods and those of *Menidia* as shorter curved rods, easily distinguished the male and female contributions to the cleavage nucleus of the hybrid eggs.

Since then a great deal of work has been done on the hybridization of many animals, and some further studies have been made on the crossing of different species of fish. In this group of animals, however, cytological observations have not been much extended. Yet G. and P. Hertwig figure a double spindle showing the portions derived from the two parents, and Miss Morris has corroborated Moenkhaus's principal findings in her crosses of *Fundulus* and *Ctenolabrus*. In the latter case, the *Fundulus* chromosomes, which are small straight rods, as Moenkhaus found them, differ clearly from those of *Ctenolabrus*, which, according to Miss Morris, are small and round.

At Woods Hole[1] in the summer of 1914 I repeated these experiments of Miss Morris on crossing the eggs of *Fundulus* with *Ctenolabrus* sperm. Since the reciprocal cross is not easily made, and since the fact as to whether the cross is possible was not under investigation, my experiments were limited to crossing in the one direction. It had occurred to me that some light might be thrown on the question of the effects of radioactivity on chromatin by treating variously with X-rays the eggs and sperm in these crosses where the chromosomes in the two differ. Experiments in four series with controls were therefore set up, and were treated with long and short exposure to X-rays as follows (adopting the series designations used by the Hertwigs in their radium experiments). Radiation treatment is indicated by *r*; untreated, or normal eggs and sperm are represented by *n*.

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[1] I am greatly indebted to the Director of the Marine Biological Laboratory for his kind assistance in providing me with the facilities of the laboratory.

Controls. *nF*♀ × *nC*♂ unradiated.
Series A. *nF*♀ × *nC*♂ radiated after fertilization.
Series B. *nF*♀ × *rC*♂.
Series C. *rF*♀ × *nC*♂.
Series D.. *rF*♀ × *rC*♂.

In general my observations corroborate those of Miss Morris as to the regular course of the events in the hybridized eggs of *Fundulus* and *Ctenolabrus*, and I find very little divergence in the behavior of the definitive chromosomes here from that described by Moenkhaus for *Fundulus* and *Menidia* crosses.

In general also, my results are in line with those of the Hertwigs in their radium experiments as to the injurious effects of the radiation upon the chromatin, shown, for example, in the failure of the egg nucleus in the series C, when strongly radiated, to take part in the development of the egg, as can be determined by the kind of chromosomes present on the spindle. Similarly, strongly radiated sperm chromatin in series B is killed and takes no part in development.

In the course of the investigation upon the radiated eggs a set of facts was discovered which bear directly upon the ever recurring question of chromosomal continuity, and which seemed to the writer, worth following out. It is the purpose of this paper to set forth those findings, and to show their bearing upon the important problem of cell structure, and to give at this time the results of the radiation experiments only very briefly, leaving them to be presented elsewhere.

During the early stages of the investigation the material studied was chiefly that which had been radiated, and most of the drawings given are from those slides. Subsequently, however, eggs of *Fundulus* which were unradiated have been sectioned, and studied from the same point of view and the conditions ascertained to be exactly the same as far as the formation of the chromosomal vesicles and their history is concerned. I wish to emphasize that neither the fact of the hybridization in the *Fundulus* ♀ × *Ctenolabrus* ♂ crosses nor the exposure to the X-radiation is responsible for the conditions here figured, for a full set of figures from normal *Fundulus* eggs in addition to those here presented could easily have been made and exactly the same conditions depicted, had the differences been thought to warrant the extra effort and time. To neither of these two experimental factors can the conditions here described be attributed.

It is quite possible and indeed the writer inclines to this opinion, that the treatment with X-rays serves to emphasize the vesicular condition of the chromosomes to make more clear relationships which undoubtedly already existed. Miss Carothers finds that X-ray treatment tends to increase the ease with which she finds chromatin granules in the peculiar vesicular condition which she calls "chromosomal vesicles."[1] It is possible that the fact that I first studied the radiated material is accountable for the recognition of the conditions here, although having once worked out the facts there I had no trouble in verifying the observations in normal untreated and uncrossed *Fundulus* eggs. Many of the findings have been confirmed by Miss Pinney in a study of *Fundulus* eggs as yet unpublished, and I have demonstrated my slides to various workers at Woods Hole, none of whom had any difficulty in seeing the conditions here described.

This account offers a rather different history of chromosome behavior from that usually given. The transition stages from one step to another are very clear, however, and do not usually permit of any other interpretation, so far as the writer can see, than the one here set forth. The new account of chromosomal formation and processes connected therewith, it will be seen, is easily fitted into the orthodox interpretation without doing violence to any important conception of mitotic behavior. It is on the details of the transition from one phase of mitosis to another that the new conception obtained from the study of the processes in *Fundulus* throws light, and at the same time offers a "raison d'être" for the appearances seen.

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[1] This use of the term is unfortunate in the light of its earlier application in the sense used throughout this paper. "Chromosomal vesicle" in the sense of Sutton and Conklin refers to a vesicular condition of the whole chromosome. Miss Carothers has subsequently pointed out that the structure she described is in reality a plasmosome vesicle. (Jour. of Morph., 28, p. 465, 1917.)

Fundulus cleavage cells show nuclei which are favorable to the study of these processes for the very reason that they are small in relation to the large amount of cytoplasm present. Because of the physical conditions thus engendered, the chromosomes and chromatic structures have a characteristic loose arrangement which permits their close study. At the same time this renders a study of individual chromosomes, as has been done for instance in orthopteran material, impossible. The behavior of a group of chromosomes as a whole is easily followed, but individual differences as in size in metaphase chromosomes are so slight that it is quite beyond the range of practicability to follow a single chromosome through the various metamorphoses it undergoes.

The material used in this investigation consisted of *Fundulus* eggs, fertilized as already stated by either *Fundulus* or *Ctenolabrus* sperm. Eggs were preserved during the early stages of cleavage (first five cleavages), and also during the later stages of development, but the cells from the early stages are the most favorable for cytological study, and it is chiefly from them that the figures are drawn. Fixation was most satisfactory in Bouin, although other fluids were also used. Iron hæmatoxylin, variously counterstained, gave the best results. The blastodiscs were removed from the yolk and embedded and sectioned separately.

Observations.

The metaphase plate may serve as a convenient point at which to begin observations on the mitotic process. Fig. 1 shows the conditions of chromosome and spindle relation in the normal *Fundulus* eggs at the time of maximum condensation of the chromosomes in the metaphase. It is from a blastomere in the fifth cleavage division. The cytoplasmic mass of such a blastomere is quite large as contrasted with the volume occupied by the spindle, and of course is large in comparison with the amount of chromatin present. This figure shows conditions similar to those which led Van der Stricht to conclude that the nuclear sap passes to the poles of the spindle and is equally divided by the ensuing halving of the blastomere. The fibers are apparently grouped into bundles leaving spaces along which the clear streams of nuclear sap may flow to the poles. Van der Stricht regards this as evidence that the sap of the dividing nucleus gives rise to that of the daughter of nuclei.

In general the events which take place during the metaphase of mitoses in these eggs at least during the earlier cleavage divisions, do not differ greatly from those occurring regularly in the majority of animal and plant cells. It is with those phases of the mitotic cycle that follow the metaphase and continue until the stage of formation of the new metaphase is reached, that are of especial note here.

Fig. 2 shows the conditions obtaining at the moment of chromosomal division. Three of a group of chromosomes are here half divided and the ends are pulling apart preparatory to their separation in the anaphase. Already it is possible to detect the chromomeres of which the chromosome is composed. The metaphase represents the stage of maximum contraction of the chromosomes. It seems that at the moment of separation the chromosome begins to loosen up. This may be interpreted to mean that their permeability increases at this time; at any rate they take in liquid (water or cell sap?) resulting in the swelling up of chromosomes and consequent separation of the chromomeres. It is in this manner that a chromosomal vesicle begins its formation. As a result of this process the chromosomes lose their property of staining densely, and until they have passed well into the telophase, the walls of the vesicles thus formed retain the chromatic stain longest. As the vesicle grows, however, it is seen that the latter characteristic is really misleading, for the walls are merely lined with very fine granules of chromatin which later become separated by the increasing growth of the vesicle.

Fig. 3 is an anaphase. The loosening up process here has brought out the true character of the chromosome. A chromosome consists of two substances, linin and chromatin, of which the former is in the nature of a sheath or sac, while the latter exists as a mass contained in the sheath. Conklin has described a similar structure for *Crepidula* chromosomes which consist of "chromatin enclosed in a linin sheath." Figs. 4 to 7 show successive steps in the anaphase and the various steps in the process of vesicle formation. The chromomeres break up and the granules of chromatin become peripherally arranged, and the center is for a while free from granules.

This central cavity of the chromosome does not seem to be a definite vacuole, as is figured for instance in some plant chromosomes, but is simply the space within the walls filled in from the fluid portion of the cytoplasm. The vesicle is achromatic in its interior due to the superficial distribution of the chromatin. Gradually there appear very fine strands or fibrils, doubtless of linin, growing from the vesicle wall inward and the granules of chromatin which accompany them are particularly noticeable at the intersection of the fibrils. Later the fibrils become stronger and somewhat reticulated, while the granules on the inner wall are farther and farther apart, and the vesicle is itself in appearance a tiny nucleus in the typical resting stage. When the reconstruction of the nucleus is complete, the granules are small and fairly equally distributed and do not stain very densely. At no time, however, does the nucleus become completely achromatic as is held in some cases. The behavior of the chromosome during its reconstruction permits a very rough comparison to an elastic bag tightly packed tightly with a fine granular substance. As the bag takes in liquid it swells up and the non-soluble granular substance becomes rolled up into tiny balls on the inner surface, and occasionally chains of the granules extend into the interior.

This interpretation of the chromosome is not unlike Conklin's view that these bodies consist of chromatin enclosed in a linin sheath. As they move to the poles they are transformed into vesicles, the interior of which becomes achromatic, "though frequently containing a nucleolus-like body, while the wall remains chromatic. These vesicles continue to enlarge and then unite into a 'resting nucleus'; the nuclear membrane is composed of the outer walls of the vesicles, while the inner walls stretch through the nucleus as chromatic portions." The formation of chromosomal vesicles in the anaphases of ova has not been a matter of infrequent observation; and this phenomenon is not commonly seen in cells other than egg cells. In other words, the formation of chromosomal vesicles is, without much doubt, correlated with the presence of an unusually large amount of cytoplasm. As the chromosomes are transformed into vesicles, they absorb, it seems, large amounts of the achromatic material from the cytoplasm, by which they grow to resemble the familiar reticular structure. Their true nature becomes masked, but as will appear later, is not really changed. Vesicular chromosomes have been described in fish eggs by Moenkhaus and certain of the figures of Miss Morris indicate that she also saw them.

According to the usual interpretation, and to this Moenkhaus subscribes, the individual vesicles fuse with their neighbors and these larger ones with each other until at last the entire nucleus is simply one great vesicle. Moenkhaus describes the transformation of the chromosomes into resting nuclei as taking place by their conversion into vesicles which during an early stage can be distinguished into two groups by the difference in size. Then the smaller vesicles "fuse at first into larger ones, giving rise to a lobed nucleus. At this state it is no longer possible to tell the two kinds of vesicles apart. The fusion continues until a single well rounded nucleus results, with all traces of its double character lost."

Increase in size of the chromosome continues through the telophase stages and to the resting stage. A rough calculation of the growth from the stage (Fig. 7) where the definite vesicular character of the chromosomes (late anaphases and early telophases) is first discernable to the full size found in resting nucleus (Fig. 12) indicates a fivefold increase. It is scarcely possible to compare quantitatively the amounts of chromatin in the condensed chromosome and in the disperse vesicular stage. But one cannot easily doubt that an actual increase has taken place in the latter case. It is during this time in all probability that the new chromatin is formed in preparation for the ensuing division; and at the same time growth in the amount of achromatic substance present would also take place. The swelling up process must of necessity be reversed at the prophase to a certain extent, and some of the liquid which had been taken in be expelled; as will be described later some shrinkage does actually take place.

During the transformation as well as subsequent to it two kinds of vesicles can be distinguished on the basis of size in the D series, in the control, and in the A series. The larger ones represent the *Fundulus* chromosomes and the smaller ones those from *Ctenolabrus*. Since in these three sets of experiments the eggs and sperm were equally injured (or in the control, were equally uninjured) by the radiation, exactly that result was to be expected in keeping with the findings of Moenkhaus and Miss Morris. The B and C series have contributed little to this phase of the investigation, but the conditions, while more complex there, apparently offer confirmation of the fact discovered by Hertwig that chromatin from radiated nuclei does not take part in the spindle to any great extent after development is initiated; or, as they have described it, the development in a strict sense thus becomes parthenogenetic. It is not to be expected therefore that the sperm chromatin in the case of the radiated B series, nor the egg chromatin in the C series will make any considerable contribution to the development of these hybrids. Such condition is found to obtain at least so far as my study has extended.

Figs. 8 and 12 represent various nuclear changes during the telophases. The cytoplasmic constriction begins to cut into the cell at about the time of Fig. 8; Fig. 12 was drawn from a cell in which the division was practically complete. There is very little difference between the latter and the resting stage shown in the next figure. During the telophase, growth of the vesicle continues and the distribution of the chromatin granules takes place. Thus the vesicles become approximated to each other so that finally there remains no space between them and each has conformed to its neighbors in assuming its final shape. Yet at all times they can be seen to retain their distinctly individual character, and in well fixed material the outlines of each can be followed. The figures are inadequate to represent the conditions for they are drawn all at one level, and the advantage that careful focusing gives in studying their structure is lost. Some of the vesicles are more distinct than others, however, as is shown in Fig. 10, where one of them stands out with especial clearness. It may not be objected to these figures that they are tangential sections and that the vesicles really fuse in the center of the nucleus, for many of these nuclei extend through three sections and in each of the sections the same conditions are seen; if this condition were due merely to the tangential cut of the section the second of a series of three would not show the vesicles separately as it actually does.

Reconstruction consists in the further swelling up and rounding out of the anaphase vesicles and their close approximation to each other in the telophase. In the completely reconstructed nucleus the approximation of the vesicles is very close, so that they are no longer to be recognized as separated bodies. Yet the appearances do not indicate any actual fusion. One sees a nucleus consisting of vesicles so closely applied to each other that they conform in shape each to the other, and there are left no spaces between them. The inner boundaries are less sharp than the nuclear membrane, but even the nuclei which are only lightly stained show areas marked off from each other which represent the tightly packed vesicles. In every resting nucleus, whether radiated or not, which I have studied these areas can be made out. Fig. 15 represents a fairly typical case, although in other nuclei the vescular structure is even more apparent. That these vesicular areas do not represent simply a delay in the complete fusion can be seen by referring to Fig. 22 where their walls are still distinct, although the new chromosomes for the next division have been formed.

The resting condition of the nuclear vesicles is shown in Figs. 13, 14 and 15. The first two of these are from eggs before the first cleavage division; until they were found the writer did not feel sure that the vesicular condition represents more than a delay in the fusion and that the final result might be such as Moenkhaus has described. Since in these cases there had been no preceding division, however, and since it does not seem that the unfused condition would persist so long as from the maturation division and over the fusion of the pronuclei if the parts do not normally coalesce, these resting nuclei gave additional credence to the belief that the vesicles maintain themselves over the resting stage as separate entities. Subsequent observations on the prophase render this position indisputable. (See below in connection with Fig. 22.) Careful study under very high power with proper conditions of lens definition and illumination on well fixed material leave very little doubt that the vesicles do remain separate. It is true that a one-to-one correspondence cannot be established between the number of vesicles in the resting stage and the number of chromosomes in the metaphase plate. This is true for two reasons: it is not possible to count with accuracy the number of these chromosomes when they are most condensed and therefore have the sharpest outline; and it is even more impossible to count the number of vesicles, for the plane of the section usually passes through some of them, and it is too uncertain to attempt to superimpose the vesicles of one section on those of the other to make a count, while at the same time all of the vesicles cannot be seen in a preparation of the nucleus as a whole. The inability to count the number of vesicles, however, is hardly an argument against their permanence for the reason that they can be followed through the interkinesis and prophases as is shown in the later figures.

These observations on the method of the reconstruction of the nuclei have a certain bearing on the problem of the nature of the nuclear membrane. Here, at least in its origin, the membrane is formed of the outer walls of the chromosomal vesicles. It may well be that the nuclear membrane of the resting cell is more complex than this, due to the interaction between nucleus and cytoplasm; indeed, some hold that the outer part of the membrane is of cytoplasmic origin, and is therefore more of an inner limiting membrane to the cytoplasm than of a constituent part of nucleus. The writer sees evidence for this view on Figs. 24 and 25 where more or less of the nuclear membrane still remains, although the inner vesicular walls have broken down and the new chromosomes have already formed.

Nuclear membranes of the *Fundulus* type which are really the outer vesicular walls find a parallel in the membrane formation around single chromosomes which have become abnormally isolated, and have grown into small nuclei distinct from the true nucleus of the cell. Such a case Boveri described for *Ascaris*. Except in regard to size and the number of chromosomes entering into them, these smaller nuclei have the same structure as those of regular types, and their membranes cannot be distinguished from that of the larger nucleus. Boveri's minute pathological nuclei are themselves an argument for the view that the chromosomes retain their independence in the normal completely fused nuclei which is usually described for this form. In these hybrid fish eggs, fundamentally the same condition obtains, for the vesicles, closely applied to each other as they are, actually retain their identity, although no cytoplasmic layer intervenes between them. Vedjdowsky has expressed a somewhat similar view in regard to the nuclear membrane for he holds that it represents the peripheral portion of the certain chromosomes that have become vacuolated in their interior.

Experimental vesiculation of chromosomes has not proven difficult to bring about in cleaving eggs. Years ago Boveri published the results on *Ascaris* eggs just mentioned, in which the chromosomes may fail to fuse and each form its own nucleus-like vesicle, and his observations have been repeatedly confirmed. Conklin's numerous experiments on *Crepidula* eggs ('12) will suffice to show how common a phenomena vesiculation is. By various means the chromosomes of the eggs were prevented from fusing and each formed in the resting stage an independent "karyomere" quite comparable to Boveri's cases. Other instances might be cited.

A phenomenon which may be so easily induced as this one is most readily understood if one thinks of the normal nucleus in interkinesis as consisting of closely crowded entities, the vesicles, derived from the telophase chromosomes, which never normally coalesce. This means that the unusual conditions of the experiment merely push them a little apart from each other. As in the case of a catalyzed reaction, a condition which already exists may be modified easily, while it is very difficult indeed to bring about an entirely new set of relationships between elements not normally reacting.

Conklin has developed the idea of karyomeres, or chromosomal vesicles, basing it upon his observations and experiments, to such a stage of completion that he can show all stages of combinations from single vesicles on up to that of the entire complex. He figures cases that very closely resemble amitosis as shown by various workers, and suggests that this superficial resemblance may be a source of error in some at least of the observations upon which the claims of the occurrence of amitosis have been made. This suggestion may be reiterated here, for it is obvious that if there should be an indention between two of the vesicles in the resting stage of a *Fundulus* nucleus, direct division would be at once called to mind.

The polarity of the cell is definitely maintained in dividing *Fundulus* eggs. As the chromosomes pass to the poles their long axes lie in the cell axis, which passes through spheres and nuclei, and the vesicles are formed with this polarity. Cytoplasmic division follows only slowly after nuclear division and the nucleus has entered upon the phase of interkinesis by the time that the cytoplasmic separation is accomplished. Also before cytoplasmic separation, the centrosome divides and each half traverses an arc of 90° in preparation for the next division. This results in the establishment of a new cell axis at the right angles to the old. Thus for a time the old and the new axes exist together, the daughter nuclei themselves marking the old and their divided centrosomes the new. Fig. 16 is such a case, the two cells shown having arisen from the result of the division of an ectodermal cell of a blastula. The line connecting the points *A* and *B* represents the axis of the parent cell, while *CD* and *EF* respectively mark the axes of the daughter cells. The fact that the two latter are nearly perpendicular to each other is of significance when it is remembered that in the next division the upper of these cells will produce the two ectodermal cells and the lower two mesodermal.

The polarity is recognizable in the vesicles in many cases, the longer axes of the vesicles in general lying in the cell axis. It is, therefore, evident that about the time of centrosomal separation the polarity of the vesicles changes. Earlier oriented with respect to the old sphere, they have now shifted to the newly established axis. Whether this is by an actual revolution of the entire vesicle, or only by a change of shape so that its short diameter now becomes its long axis is not clear, but it seems probable that the former process takes place. When condensation occurs and the new prophase chromosomes are formed they show the polarity in a very striking manner, almost every chromosome in the spireme stage being oriented in the long axis of the forming spindle (see Fig. 22). This of course is to be expected in view of the definite polarity of the vesicles in the prophase as shown in Fig. 17.

In reconstructing nuclei where the fixation is not quite perfect, the walls of the vesicles sometimes cannot be seen. They are very delicate and require a very complete fixation if they are to be preserved in their entirety. When the nucleus enters upon the first steps of the prophase and liquid is extruded from the vesicles so that they shrink or condense, the walls are particularly difficult to preserve. This results in an artificial running together of the granules into irregular clumps. Yet the area formerly occupied by the vesicle is still to be seen separating one clump of chromatin from the next, and the nuclear membrane is still retained although in a shrunken condition. The chromatin so massed by the irregular plasmolysis of the walls of the vesicles in some instances comes to resemble a prophase chromosome. Mere clumps of chromatin granules, however, with nuclear membrane intact and the inner vesicle walls gone indicate artifacts rather than normal conditions. Yet even they serve to suggest certain facts which are represented in the normal nuclei.

The new chromosomes arise within the old vesicles. When the writer first began the study of this phase of the subject, he was led to expect that the shrinkage which takes place at the beginning of the prophase as shown in Figs. 13, 17, and 18, would continue and the formation of the new chromosomes would be simply the reverse of the process by which the vesicles had been formed. Such is not the case, however, for it is now certain that the new chromosomes arise endogenously. The formation begins within the vesicle with the gradual increase in size and the aggregation of the chromatin granules upon its walls. The typical condition of the vesicles during rest shows small granules distributed fairly uniformly throughout it. Succeeding this stage of general chromatic dispersion the vesicles appear to shrink, expressing part of their water content; this phenomenon is manifested doubly, first by the clear non-fibrillar area about the nucleus, the extent of which before shrinkage is thus marked, and second by the concentration of the contents of the vesicle, which in fixed material appears denser and takes more orange G than before. Coincident with this change the amount of chromatin appears to increase and the aggregation of the granules takes place, the larger masses of chromatin appearing along the periphery and perhaps extending into the center along a single thread. The aggregation continues until masses the size of the chromomeres of the anaphase are formed and have become arranged in a linear series. The linin sheath about this linear aggregation of chromomeres forms early. This structure is the new chromosome and must be thought of as chromosomal in nature quite as much as the more familiar metaphase stages. When it is completely formed the walls of the vesicle may be seen to be gradually dissolved and the denser contents to diffuse into the cytoplasm. About this time or a little later the split of the new, or filial, chromosome takes place, each chromosome and chromomere showing it about equally.

Figs. 17 and 18 show the first sign of the next division. The chromatin granules are well dispersed and the vesicles definitely oriented, while the pre-mitotic shrinkage has taken place. In Fig. 19 the aggregation of the chromatin granules has begun and the vesicles in this state can always be recognized as about to divide. In Fig. 20 is seen the linear arrangement of the chromomeres, a process which is well advanced in Fig. 21. In this latter case indeed the chromosomes are well formed and the linin sheaths can be made out. In these figures the walls of the vesicles are not as clear as in some cases, but the denser gel-like portion still shows the form and relationships of the vesicles. There would seem to be some variation as to the exact time when the disappearance of the walls takes place, in relation to the development of the spindle structures at least.

Figs. 21 and 22 are prophases in the spireme stage, early and later. Fig. 22 is an especially clear case showing the newly formed filial chromosomes while at the same time the walls of the old vesicles have not yet disappeared. In the upper part of this figure they are less distinct, but elsewhere the vesicles are easily seen in their entirety. The walls stand out clearly and take the orange stain strongly, so that there can be no mistake about their presence. The spheres are not shown in the plane of this section, but the spindle fibers indicate the polarity with most of the chromosomes oriented in the spindle axis.

Of the greatest importance is the evidence presented in Fig. 22. The presence in the vesicles, the walls of which are still intact, of the new filial chromosomes, for these spireme structures are now definite chromosomes, is the crux of the argument for chromosomal continuity. It is not difficult to follow through the history of the vesicles from the time of their earliest formation out of the metaphase chromosomes thorugh anaphases, telophases, and interkinesis, and to see that the vesicle represents, so far as the chromosomal bodies are concerned, a continuous unit of structure. The parental chromosomes are not lost in the vesicle stage; they are merely metamorphosed, appearing in a new form and the chain of the continuity is never broken. In the vesicles the continuity is still maintained throughout the resting period of the nucleus and it is only in the late prophase of the daughter generation that the vesicular walls disappear. But before that disappearance, the new filial chromosomes have been formed and are in the so-called spireme stage, representing uninterruptedly the persistent continuity of the units with which the cycle started in the parental metaphase. In this spireme stage the chromosomes are as definitely chromosomes as in the metaphase which immediately succeeds, and they have only to condense further and to attach themselves to the spindle to enter the metaphase, a process obviously favored by the dissolution of the nuclear walls.

In Fig. 23 all traces of the vesicle walls have disappeared and the chromosomes resemble the late prophase chromosomes of many animals. This is the only case in which the longitudinal split is seen, and it may be that it is really precocious, or that the figure represents the facts only in part. In most nuclei in which the chromosomes are well formed the spindle development has progressed farther than in this case. These split chromosomes are suggestive, however, when compared with such chromosomes as shown in Fig. 2.

The manner of attachment of the fiber to the chromosomes and the steps leading to the metaphase plate condition are shown in Figs. 24 and 25. Fig. 24 is an oblique section in which there is only one sphere. The walls of the vesicles at the polar end of the nucleus are gradually disappearing and the fibers are making their way to prophase chromosomes; traces of the walls however can yet be seen. In the next figure the attachment of the fibers is practically completed and the chromosomes have become definitely placed upon the spindle. While in general they were orientated in the prophase with their long axis in the spindle axis, they become definitely "drawn" into place with the attachment to the fibers. The condensation of the chromosomes and their final arrangement on the spindle completes the cycle and brings us again to the stage shown in Fig. 1.

These last two figures are interesting also for their likeness to the conditions found by Van der Stricht in the egg of *Thysanozoön*. In this form the spindle and its fibers can be differentiated into several parts. The principle cone, "cone principaux," of fibers corresponds to the mantle fibers and is composed of those which attach to the chromosomes themselves. Just outside these but still in the area of the nucleus is a cone of fibers which do not attach; this Van der Stricht called "cone accessoire." Finally out in the cytoplasm is to be seen a series of the fibers which interlock with those from the other end of the spindle; these are the "fibers cytoplasmique." In *Fundulus* eggs exactly the same conditions seem to prevail (Figs. 25 and 1).

As the central spindle fibers make their way to the chromosomes and attach to them there is a gradual movement of the chromosomes to the equator of the spindle to form a plate. During this movement the last stages in the condensation of the chromosomes takes place, resulting in the formation of the densely staining metaphase rods, such as were figured by Moenkhaus and Miss Morris. As they condense the chromosomes increase in thickness, so that the metaphase chromosome is both shorter and broader than that of any other stage. The nuclear sap also becomes completely diffused into the cytoplasm by this time, and no distinction can be seen. The final event leading up to the formation of the plate is a change in position of the chromosomes with the reference to the axis of the spindle. Previously oriented with their axes parallel to that of the spindle, they now come to lie at right angles with their free ends pointing towards the periphery. When this stage has been reached the plate is fully formed.

Thus it is possible to follow out the history of the chromosome group in the early cleavages of *Fundulus* without a single break in the continuity from the metaphase of one generation to that of the next. It should perhaps be pointed out again that this account refers to no particular mitosis. In all of the early cleavage divisions in which the nuclear structures are large enough for study the facts are as here related.

Since making the above observations on *Fundulus* the writer has been able to extend them to many stages of the eggs of *Corregonus albus*, and to satisfy himself that the behavior of nuclear structures is essentially similar in both fish.

Figs. 26 to 29 do not add any direct proof to the theory of the continuity of the chromosomes, but they serve to throw a side light on the process of spindle formation, at least as it occurs in the gastrulæ of this form. The writer has not seen the same conditions in the early cleavage cells and does not presume to claim that they occur there, but since the gastrula shows these rather unusual phenomena, they may be set forth here to assist perhaps in the interpretation of similar appearances elsewhere. At the beginning of the prophase, the formation of the central spindle takes place between the centers that are here divided and are passing to the opposite side of the nucleus. This process, it will be noted, occurs at a relatively later period in the division of the gastrula cells than in the earlier cleavage cells. Fig. 27 is a face view of a spindle of this type a little farther developed; certain of the chromosomal vesicles become separated to make way for the developing spindle which gradually sinks down between them and is thus ready for the attachment of the fibers to the chromosomes whenever the latter are formed. It might be thought by analogy with such forms as *Crepidula* that

Fig. 27 really represents the male and female halves of the nucleus. That this is not the case, however, is shown by Fig. 28 in which only the *Fundulus* chromosomes are visible. Miss Morris found that in *Fundulus* cytoplasm the *Ctenolabrus* chromosomes are small round bodies, distinctly differentiated from those of *Fundulus*. This observation I can corroborate; the cytoplasm modifies the character of the foreign chromosomes, and they can be recognized as different from those of the egg. The egg from which these four figures were drawn was fertilized with *Ctenolabrus* sperm that had been subjected to X-radiation for fifteen minutes; this is sufficient duration of time for the radiation to kill the male chromatin, which therefore made no contribution to the development of the egg and is not represented, as is shown by Fig. 28 which contains only *Fundulus* chromosomes. Since there is no male contribution to this nucleus, the interpretation that Fig. 27 shows merely the male and female halves of the nucleus is not valid. Rather the true state of affairs is made clear by Fig. 29 which is a cross-section of such a nucleus as is shown in Fig. 27; the central spindle makes its way through the groove shown on the upper side of the nucleus.

Discussion.

(*a*) *Chromosomal Vesicles.*

While there are numerous cases cited in the literature which would seem to give the conception of the constitution of chromosomes and resting nuclei here set forth a fairly wide application, the writer does not care to attempt a generalization from these observations extending the principle to other forms, although some of the cases which support these findings must be given. Any generalization at this time would seem unwarranted for too many instances are known where neither the chromosomes nor the chromatin during rest seem to answer exactly to these descriptions. There are many cases, for example, in which the chromosomes, judging from their behavior in the prophases, may be regarded as made up in chromatin granules strung upon a linin thread, and it is even possible to identify the granules in homologous chromosomes. How such a structure is to be derived from a permanent vesicular chromosome is not yet clear, although this derivation is perhaps thinkable. The account here given aims rather merely to present the conditions as they are found in the fish eggs which I have studied, and to point out that these facts may have significance for current cytological and genetic speculation.

Conklin in his studies on *Crepidula* (1902) has perhaps followed out the history of the chromosomal vesicles, or karyomeres, as he calls them, more completely than any other observer. Of the behavior of the *Crepidula* chromosomes he says: "In large cells where the divisions succeed one another at short intervals the chromosomes begin the growth characteristic of the daughter nuclei, the absorption of substances from the cell body, before they have fused together, whereas in small cells or cells which divide only at long intervals the chromosomes fuse before the absorption of achromatic material begins."

"The history of the nuclear changes during the cycle of division may be summarized as follows: (1) The chromosomes, consisting of chromatin enclosed in a linin sheath, divide and move to the poles of the spindle where they partly surround the spheres. (2) Here they become vesicular, the interior of the vesicle becoming achromatic, though frequently containing a nucleolus like body, while the walls remain chromatic. (3) The vesicles continue to enlarge and then unite into the 'resting nucleus'; the nuclear membrane is composed of the outermost walls of the vesicles, while the inner walls stretch through the nucleus as chromatic partitions; the chromosomal vesicles from the egg and sperm nuclei remain distinct longer than those from the same nucleus. (4) The chromatin of the inner alveolar walls then aggregates into threads, giving rise to a 'chromatic reticulum,' though the linin still preserves, for a time at least, the alveolar structure. (5) The chromatin of these threads then separates into spherules, which are connected together by linin threads; these spherules vary in size, and at first are solid, and stain alike. (6) They become hollow and are differentiated into oxy- and basichromatin. (7) In the first maturation, each of the basichromatin spherules, or bodies, grows into an individual chromosome; in the cleavage, the basichromatin spherules unite into several linear series, thus forming a segmented spireme.

(8) The oxychromatin spherules grow smaller and are dissolved in the nuclear sap while others are arranged in series on the linin threads into which they are formed; these threads with attached spherules form the spindle fibers." Thus the relation of the old vesicle to the new chromosome is followed out.

Evidently from this description there is a close parallel between the behavior of the chromosomes of *Crepidula* and those of *Fundulus* eggs. Much of this summary will fit, word for word, the conditions which have been delineated in the preceding pages, although in *Fundulus* the vesicles do not fuse in the resting stage.

Conklin points out the fact that "such vesicles are found generally, if not uniformly, in the early divisions of the ova, though they are not usually found in other mitoses." He attributes this to the difference in size and rapidity of division of the blastomeres as compared with tissue cells, and concludes "that in the large cells where divisions succeed each other at short intervals, the chromosomes begin the growth characteristic of the daughter nuclei, *i. e.*, the absorption of substances from the cell body, before they have fused together, whereas in small cells or cells which divide only at long intervals the chromosomes fuse before the absorption of achromatic material begins." With this conclusion the facts as found in *Fundulus* in general are in accord.

In both kinds of eggs also the growth stages of the daughter nuclei are quite alike. In *Crepidula* "after the fusion of the chromosomal vesicles to form the daughter nuclei, the latter continue to absorb achromatic material, growing larger and larger until the prophase of the next division. A part at least of the achromatic material absorbed is derived from the sphere which in turn contains interfilar substance of the spindle and aster. This recalls the conclusions of O. Hertwig in which he points out that in the formation of the daughter nucleus the chromosomes absorb 'Kernsaft' and become vesicular, the process being the reverse of what occurs in the beginning of division where 'Kernsaft' is set free into the cell body. A similar view was held by Butschli . . . ."

The most important point of difference between Conklin's observations and mine is the matter of ultimate fusion of the chromosomal vesicles in the resting stage, and the formation of the new chromosome in the vesicle. In *Fundulus* there can be no doubt that a direct continuity exists between vesicle and prophase chromosome and that the vesicles do not fuse during the rest period. One is compelled to suspect that *Crepidula* offers less favorable opportunity for the study of this particular detail than *Fundulus.* Conklin himself believed that there is a real independence of the vesicles although they appeared to fuse, and it may be inferred from the following paragraph that he expected their persistent identity throughout the resting nucleus to be proven in time. The observations on *Fundulus* may be taken as a confirmation of his view expressed in 1902.

"It sometimes happens, especially in eggs in which more than the normal number of centrosomes and asters are present, that some or all of the chromosomal vesicles do not fuse but remain distinct through the whole resting period. In such cases each of the vesicles behaves like a minature nucleus, absorbing the achromatic material and forming a network of chromatin either within the vesicle or on its walls. In this growth and differentiation the vesicle keeps pace, step by step, with the normal nucleus, so that one must regard the resting nucleus as virtually composed of vesicles, though their union may be so intimate as to hide this structure. The resting nucleus is not, therefore, a single structure any more than is the nuclear plate. It is composed of units each of which so far as known, has the properties of the entire nucleus, and the union of these vesicles into a single one may be considered as a secondary character. It is altogether probable that the chromosomes and hence the chromosomal vesicles, preserve their identity throughout the resting nucleus."

Moenkhaus also was unable to see in the nuclei of the resting cells evidences of separate chromosomes, yet he too, believed that the substance of each chromosome forms a persisting unit. We may expect then that the next step in the proof of chromosome continuity and persistence is the recognition of structures in the resting nuclei which can be homologized with the chromosomes of the metaphase plate. This I believe is accomplished in the fish eggs of my experiments. Moenkhaus states the case as follows: "The question whether the individual chromosomes persist through the resting stage so that upon the resolution of the reticulum into the chromosomes the same component chromatin granules again go together to make the same chromosomes from which they were derived is a question first raised by Rabl and later definitely stated by Boveri. Since that time so much evidence has accumulated to support this conclusion that it has come to be rather generally accepted. Even a general review of the evidence is unnecessary here. Such a review would show that the fact has never been definitely demonstrated. Some of the most direct evidences yet given are the observations of Herla and Zoja on the *Ascaris* hybrids in which it was shown that the small chromosomes of the variety *univalens* which entered the resting nucleus with the larger ones of the variety *bivalens* again emerged in characteristic form. Equally strong evidence is now afforded by my observations on hybrid fishes. Here, as in the *Ascaris* hybrid, two kinds of chromosomes enter the resting nucleus from which each kind again emerges. As long as the two kinds remain grouped, as during the two divisions, this fact has little added significance, since within each group it would be perfectly possible for the component chromosomes to exchange chromatin granules during the resting period. If, however, as occurs in the later cleavages, the two kinds of chromosomes become mingled the chromatin granules of both kinds must lie mingled together within the resting nucleus. If from a nucleus the two kinds again emerge, it amounts almost to a demonstration that the chromatin substance of a given chromosome forms a unit and that the unit persists."

The evidence presented by Conklin and by Moenkhaus most nearly touches the subject of my investigation of any that I have found in the literature. Both of these workers failed to see in their material that the chromosomes may actually be discerned in the nuclei during rest as distinct vesicles. Yet both believed that a continuity of structure does exist and that the chromosomes form persistent, independent units. My material offers more complete and direct evidence that the vesicles persist and leaves little room for doubt that the conclusion of the observers mentioned is the correct one.

Sutton (1900) was the first of a group of cytologists working on the chromosomes of grasshoppers to point out that in the early stages of nuclear formation each chromosome forms a vesicle about itself, but thought that later the proximal ends of these fused, a condition which he interpreted as supporting the idea of chromosome individuality. His conception of a chromosome is not unlike that of Conklin, and has since been amply confirmed.

The occurrence of the chromosomal vesicles is quite common in the eggs of animals, and it has also been reported in certain other tissues as well. The following list is presented merely to give a few examples; it is in no way complete, but will serve to show the wide distribution of the phenomenon.

Name, W. G. Von, Trans. Conn. Acad., Vol. X., 1899.	*Planorbis*, early development of eggs.	Figures vesicles in anaphases.
Goldschmidt, R., Zeit. f. wiss. Zool., Bd. 71, 1902.	*Polystomum* eggs.	Karyomerites, at first one to a chromosome; later fuse and are not recognizable.
Von Kemnitz, G. A., Arch. f. Zellf., Bd. 10, 1913.	*Branchycœlium* eggs.	Karyomerites which later fuse.
Grille, K., Arch. f. Zellf., 12, 1914.	*Gyrodactylus elegans* eggs.	Karyomerites which fuse later.
Boveri, Th., Merkel u. Bonnet's Ergebnisse, 1891.	*Ascaris* eggs.	Accidentally separated chromosomes produce vesicles, from which new chromosome arises.
Lillie, F. R., Jour. Exp. Zool., 12, 1912.	*Nereis* eggs.	Figures vesicles in telophase; later fuse in reconstruction.
Mead, A. D., Jour. of Morph., 13, 1897.	*Chætopterus* eggs.	Figures show what are probably chromosomal vesicles.
Lefevre, G., Jour. Exp. Zool., 4, 1907.	*Thalassema* first cleavage.	Vesicles in anaphases; later they fuse.
Wilson, E. B., The Cell, 1900.	*Toxopneustes* egg cleavage.	Figures vesicles in telophase.
Boveri, Th., Zellstudien, IV., 1900.	*Echinus* eggs.	Figures vesicles in anaphase.
Buchner, P., Arch. f. Zellf., 6, 1911.	Sea urchin.	Karyomerites in first parthenogenetic maturation division.
Konopacki, M., Arch. f. Zellf., 1911.	Sea urchin.	Vesicles produced experimentally; thinks of them as nuclear budding.
Schaxel, J., Arch. f. Mikr. Anat., Bd. 76, 1911.	*Strongylocentrotus* egg.	Vesicles in telophase; they fuse, then alveolize, producing fine nuclear network.
Bury, J., Arch. f. Entw., 36, 1913.	Echinoid egg cleavage.	Karyomerites in early stages.

Smallwood, M., Morph. Jahrb., 33, 1905.	Nudibranch eggs.	Telophase vesicles which later fuse.
Lillie, F. R., Jour. Morph., 17, 1901.	*Unio* eggs.	Figure of resting nucleus yet in reconstructing process shows vesicles.
Conklin, E. G., Jour. Phila. Acad. Nat. Sci., 15, 1912.	*Crepidula* eggs.	Experimentally produced karyomeres from single chromosomes.
Häcker, V., Arch. f. Mikr. Anat., 46, 1895.	*Cyclops* eggs.	A few small vesicles which are male and female contributions.
Ruckert, J., Arch. f. Mikr. Anat., 45, 1895.	*Cyclops* eggs.	Chromosomal vesicles in both male and female halves, in transition to resting stage.
Kühn, A., Arch. f. Zellf., 1, 1908.	*Daphina* parthenogenetic eggs.	Vesicles in telophase, but they do not prove continuity for they are unrecognizable during rest.
Amma, K., Arch. f. Zellf., 6, 1911.	*Cyclops* eggs.	Figures vesicles in four-cell stage.
Bigelow, M. A., Bul. Mus. Comp. Zool., XL., 1902.	*Lepas* eggs.	Figures show vesicles in early cleavage stages.
Sutton, S. S., Kans. Univ. Quart., 9, 1900.	*Brachystola* spermatogenesis.	Chromosomal vesicles which became fused at one end.
Otte, H., Zool. Jahrb., 24, 1907.	*Locusta* spermatogenesis.	Chromosomal vesicles which were thought to remain distinct.
Davis, H. S., Bul. Mus. Comp. Zool., 53, 1908.	*Locustidæ* and *Acrididæ*, spermatogenesis.	Vesicles fuse at one end.
Pinney, E., Kans. Univ. Sci. Bul., 4, 1908.	*Phrynotettix* spermatogenesis.	Vesicles which were thought to remain distinct through resting stage.
Wilson, E. B., Jour. Exp. Zool., 13, 1912.	*Oncopeltus* spermatogenesis.	No rest between maturation divisions; chromosomes crowd together, like vesicles, without loosening up; never fuse.
Wenrich, D. H., Bul. Mus. Comp. Zool., 60, 1916.	*Phrynotettix* spermatogenesis.	Vesicles retain their own limits in the nucleus in rest.
Medes, G., Biol. Bull., 9, 1905.	*Scutigera forceps* spermatogenesis.	Vesicles in second spermatocyte; they fuse; but traces are often long retained.
Conklin, E. G., Jour. Phila. Acad. Nat. Sci., 13, 1905.	*Cynthia* eggs.	Vesicles in cleavages.
Moenkhaus, W. J., Amer. Jour. Anat., 3, 1904.	Eggs of *Fundulus* and *Menidia* crosses.	Vesicles which fuse, but believed to be distinct though invisible as such in rest.
Morris, M., Jour. Exp. Zool., 16, 1914.	*Fundulus* and *Ctenolabrus* crosses.	Vesicles which fuse.

Hertwig, G. & P., Arch. f. Mikr. Anat., 84, 1914.	*Gobius* and *Crenilabrus* crosses.	Vesicles in telophases which are thought to be signs of degeneration.
Van der Stricht, O., Arch. de Biol., 12, 1892.	*Triton* eggs.	Vesicles formed by looping of chromosomes; figures of resting nuclei suggest traces of vesicles.

(*b*) *Chromosomal Continuity.*

Chromosomal individuality has been so often argued of late years that a renewal of the discussion seems an almost trite and needless repetition. However, in the light of the facts here brought forth it may not be out of place to review the present status of the question. It must be understood that no argument is made that chromosomes are directly transmitted from one cell generation to the next as *identities;* that conception has long since been abandoned for it has been shown to be inadequate. Rather the argument is that the new chromosomes arise from the same structural substance as the old, a theory of genetic continuity. One prophase chromosome and only one arises from the substance of a single one from the preceding division. It is such a theory which is the subject of this discussion.

The hypothesis of chromosome individuality dates back to Rabl ('85) who "concluded that the chromosomes do not lose their individuality at the time of division, but persist in the chromatic reticulum of the nucleus" (Wilson). He thought that the disappearance of the chromosome in the reticulum was only apparent, for he could recognize in the reticulum of the salamander nuclei portions corresponding to the chromosomes. The reticulum is formed by the outgrowth of processes, secondary and tertiary, from the chromosomes which while fusing on the neighboring chromosomes, nevertheless, maintain their own identity.

That Rabl's view regarding the persistence of the chromosomes has not found general confirmation is indicated by the following quotation from Wilson, who, in discussing the theory of chromosome individuality[1] remarks "that in vast majority of cases the identity of the chromosome is wholly lost in the resting nucleus and the attempts to identify them through the polarity or other morphological features of the nuclear network have, on the whole, been futile. It is, therefore, an abuse of language to speak of a persistent individuality of the chromosome."

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[1] The Cell, p. 300.

There is, however, a great mass of evidence in favor of a view of chromosome individuality or rather, since there is so little evidence of actual chromosome persistence as unchanging and identical bodies during the resting stage, the hypothesis of genetic continuity of chromosomes. The fact that almost invariably the same number of chromosomes emerges from the resting nucleus that went into it, Boveri's studies of abnormal variations in the early development of *Ascaris* eggs, Zur Strassen's observations of the giant embryos of *Ascaris*, the previously mentioned discoveries of the independence of maternal and paternal chromatin in hybrid eggs, the constant recurrence of chromosomes in the same form and even position, *e. g.*, in root tip cells of *Yucca* (Clemens Müller) and in *Ascaris* eggs (Boveri), their gradation in size invariably repeated in certain insect germ cells, the history now well known of the accessory chromosome, which retains its identity from one cell generation to the next, the recent studies which have shown clearly the homologies of chromosomes other than the accessory as well as their independent behavior, and finally the correlation of the results of recent studies in genetics with such a behavior on the part of the chromosomes, all these points constitute strong inferential evidence in favor of a theory of chromosome continuity.

Chromosome continuity is a theory as applied to resting nuclei only. In the mitotic stages the facts are clear; the chromosomes are distinct bodies which behave as units and it is a general observation that those of one cell are homologous with those of others in the same organs and in general in the same species. It is only in the interkinesis that the facts partake in any way of the nature of a hypothesis and admit of differences of interpretation regarding the point here discussed. In this state it is difficult or impossible to recognize chromosomes as distinct entities and accounts which seek to distinguish them throughout rest have been and must be received with great caution. Adverse criticisms of the theory have been based upon claims of faulty technique, faulty observation, upon obvious objections to a hypothesis of strict individuality (which is impossible both in a chemical sense where two phases exist in the same cell, and where there must be interaction between the constituent parts of the system, and in a mathematical sense of unchangeableness) and to the more deeply grounded objection that the process is one of dynamic character and is not subject to morphological explanation.

Mathew's objection is perhaps the most significant of the first group. He says "All so-called nuclear stains of basic nature except the mordanted stains as iron hæmatoxylin combine with nucleic acid. Cytologists in following chromatin and chromosomes may be following only the inert skeletal material of the nucleus while the active albuminous material is entirely neglected since it does not gel or stain with basic dyes." Yet significance must attach to the *constant recurrence* of these structures whether they are of skeletal nature or otherwise, and to the fact that chromosome structures reproduce themselves and have done so indefinitely. If they are not more than skeletal in nature, they are at least the manifestations of the "protein or basic" and perhaps more active parts of the cell, and the correlation is so close that their behavior is no less significant.

Fick's objections as Wilson has pointed out refer only to the strict interpretation of individuality which is not supported by the evidence and which is no longer held by cytologists in general.

With regard to the morphological method of attack as one means of studying dynamic problems more is to be said in defense than against. The nucleus is, indeed, "a dynamic system," as one of the chief critics of the continuity hypothesis has pointed out; and the process by which it reproduces itself is of all biological phenomena the best example of a dynamic process. Yet a dynamic process whose basis is not material, indeed not morphological, is only with difficulty thinkable. In whatever may be the final terms of our thinking, chromosomes, chromomeres, molecules or ions, we deal in the end with structure and configuration; and this fact must make us hesitate in any attempt to detract from the significance of chromosome structure and continuity. We may believe consistently that the chromosome is a continuing structure, and that its correlations with processes of heredity are even so close as to be causal, without forgetting that its functions and behavior are dynamic. Child has pointed out with great propriety that "instead of being the basis the chromosome is itself a problem of heredity." We no longer think of chromosomes as ultimate units of structure or the unreducible cause of heredity processes. It is true that the heredity processes have been merely pushed farther back into the cell for explanation; but even then, a distinct step has been taken if the correlation can be traced as in *Drosophila*, between body characters and chromosomes; and a more complete dynamic explanation of the processes, along with that of chromosome structure and behavior, can only be eagerly awaited. Dynamic processes are of course at the basis of all biological functions; with the physical structure of the organic body they do their work, and new facts, if real facts, must be comprehended in the larger dynamic system. Biology is now well launched out in the attempt to find dynamic solutions to its problems, and morphology including cell morphology still has contributions to make to the attempt. It is difficult indeed to understand the structure of a mechanism without some knowledge of its function, but it is equally difficult to appreciate a structureless function.

Of interest from the standpoint of chromosomal independence are the observations now being carried on by Chambers on the micro-dissection of the living cell. It appears from this work that there is gradually being established from the living material a confirmation of the general principles of cell structure and behavior as worked out on fixed preparations. The fact that certain structures as polar radiations, are not visible in the living cell and are present in fixed cells, perhaps due to injury or precipitation caused by the fixing process, is not an argument against their significance since they occur constantly and in precise relations to other structures. If not significant in themselves they are at least manifestations of processes which lie more deeply in the cell. Of particular interest here is the observation as yet unpublished of Dr. Chambers, of which he kindly permits mention to be made here, that in grasshopper spermatocytes the telophasic chromosomes are to be found in the vesicular condition. When single grasshopper chromosomes are removed and allowed to grow in plasma they develop into round vesicles which by puncturing with the needle can be made to collapse.

From the work of many cytologists there has gradually accumulated a great mass of inferential evidence for chromosomal continuity which is well known, and the general nature of which was mentioned earlier in this paper. The form and size relations of chromosomes, the behavior of the sex chromosomes, which can be followed from one generation to the next, the homologous character of somatic chromosomes, certain chromosomes not correlated with sex (according to the work reported by a number of investigators, *e. g.*, by Wilson and by Carothers), all of these facts can be understood only with the greatest difficulty on any other basis than that of continuity of structure. A very recent case is Wenrich's finding that in *Phrynotettix* there exists a pair, "B," whose architecture is constant for all individuals studied, and another pair, "A," which is recognizable "through all stages from spermatogonia to spermatids." This case is very clear and of great importance.

Vejdovsky's ingenious theory is of interest in relation to my observations, although I do not find that the chromosome behavior of *Fundulus* tallies with his descriptions of observations upon which he bases his theory. He holds that the old chromosomes produce anlagen from which the new ones arise. As each organic individual comes from the anlage in the female organs, so do the chromosomes also arise from anlagen. The old chromosomes produce only anlagen for the new generation and the formation of the latter sets in with the beginning of the new cell. This anlage is the chromonema which gives rise to the two substances of which chromosomes consist, chromatin and linin. Each chromosome begins its existence with a spiral chromatin thread while the linin substrate of the old thread is lost to the anlage of the "Kernenchylem."

Reference must be made to the bearing of genetical work on the doctrine of the significance of chromosomes. The results of the great mass of recent work in this field demands as a practical necessity that chromosomes be treated as if they are actually persistent individuals, and the genetical behavior of heredity characters is being steadily brought into line with chromosomal behavior as a more thorough-going knowledge of the latter is obtained. It is a noteworthy fact that those cases where heredity does not seem to conform to the expectation based upon the behavior of chromosomes are usually cases where the chromosomes are small, numerous and imperfectly known, and that in general the difficulties in comparing the two sets of facts have to a large extent disappeared with increasing knowledge.

The significance of hybridization experiments for the doctrine of chromosome continuity has been repeatedly pointed out, and a single case will serve to illustrate it. Federley crosses different species of the moth *Pygæra* in which the diploid and haploid chromosome numbers are as follows: *P. anachoreta*, sixty and thirty; *P. curtula*, fifty-eight and twenty-nine; and *P. pigra*, forty-six and twenty-three. In whatever cross was made of these three species the chromosome number of the hybrid was the sum of the two haploid sets that went in. According to Federley the chromosomes preserve their continuity through many generations of foreign cytoplasm.

Evidence that there is chromosome continuity in plants is quite abundant, as may be seen for example, from the reviews of Stout and of Wenrich. Only the main trends of the evidence need be mentioned here. First of these are the findings of Rosenberg, Overton, Lundagård and others that in the somatic cells of a considerable number of plants there occur in the resting nuclei chromatic bodies of the same number as the chromosomes during metaphase: these bodies enlarge directly into the prophase chromosomes. To certain other types of plant nuclei the views of Gregoire and his school apply. According to this idea the chromosomes as they pass to the poles become vacuolated, or alveolized, often forming in the resting nuclei reticulate bands which later are transformed into a spiral thread, the prophase chromosome of the next division. Of especial interest because of the very suggestive similarity of the method by which chromosomes are formed in *Fundulus*, is the view of Bonnevie resulting from the study of both plant and animal cells, *Allium* and *Ascaris*. She believes that there is genetic continuity of chromosomes although there is no *identity* between those of different mitoses: from the substance of each chromosome at the end of its life however, there arises endogenously the new prophase chromosome. The old chromosome is an earlier existing "endogen" for the foundation of the new. She regards the chromatic substance as the persistent continuing portion, while the achromatic substances between cell generations are lost. My observations on *Fundulus* bear out the main facts of this conclusion, although the details of the process differ.

It remains finally to inquire whether any significance attaches to the fact that the conditions described in this paper occur in tissues that are dividing rapidly. In all cases where persistent chromosomal structures are found the period of interkinesis is comparatively short; the question may be raised therefore whether in cells in which the interkinesis is of long duration the fusion of the vesicles is not more complete and the continuity perhaps lost. It seems to the writer that this is probably not the case, although the latter cells may require a more delicate technique and more close observation to discover that the vesicles are persistent entities. The fact that there is no resting period intervening between the maturation divisions and that the chromosomes in this case persist from one mitosis to the next in itself argues for the view of continuity elsewhere. We may look upon this discrete nature of these chromosomes as a manifestation of a condition that is general for cells and is merely emphasized in this particular case. A somewhat similar principle is that of a catalyzer, which is not able to initiate a reaction, but merely to hasten one that ordinarily takes place at a much slower rate. A second reply to the question of the significance of the above mentioned fact is that usually cells which divide very slowly are not of hereditary importance since those cells which bear the hereditary qualities usually divide with fair rapidity.

Summary.

The chromosomes in the eggs of *Fundulus* can be traced from the metaphase of one cell generation through all the stages of mitosis and interkinesis as continuous structures which give rise to the prophase chromosomes of the next mitosis. As they pass toward the poles in the anaphase they gradually loosen up, showing their constituent chromomeres, and finally form vesicles which come together in the telophase. Although they are closely applied to each other in the resting nucleus, they still maintain their unity of structure. When the nucleus begins the next division the new chromosomes are produced endogenously, each within the substance of one of the old vesicles which persists until the new chromosome is well formed. Thus there is clearly a continuity of substance from the old to the new metaphase chromosome. These observations establish clearly the genetic continuity of the chromosomes for this case and the evidence in general is so strong that the fact may be held as of wide application. The preceding account gives in detail the facts as observed in the mitoses of *Fundulus* eggs, a general summary of the occurrence of chromosomal vesicles and the relation of other cases to the present one, and finally a general statement of the present status of the theory of genetic continuity of chromosomes with the main points of evidence for it.

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EXPLANATION OF PLATE I.

These figures were all drawn with the aid of a camera lucida and Leitz two mm. objective with twelve compensating ocular (except figure sixteen as noted below). They are reproduced here at a magnification of a little more than eighteen hundred diameters.

FIG. 1. F♀ × F♂ normal; fifth cleavage division. Metaphase plate of which the chromosomes of two levels only are shown. Stage of maximum condensation of chromosomes.

FIG. 2. F♀ × C♂. Both radiated for three minutes before fertilization; second cleavage division. The beginning of chromosome division and first signs of separation of chromosomes are shown in this part of metaphase.

FIG. 3. F♀ × C♂; eggs radiated for fifteen minutes before fertilization; second cleavage division anaphase. The loosely formed chromosomes show increased size, the linin membrane and the chromosomes.

FIG. 4. F♂ × C♂; sperm radiated for fifteen minutes before fertilization; second cleavage division; chromosomes in similar condition to Fig. 3.

FIG. 5. F♀ × C♂; sperm radiated for three minutes before fertilization. The beginning of the vesiculation of the chromosomes. Liquid is being taken into the linin sac which in consequence swells up. A few of the chromosomes from both ends of the anaphase spindle are shown.

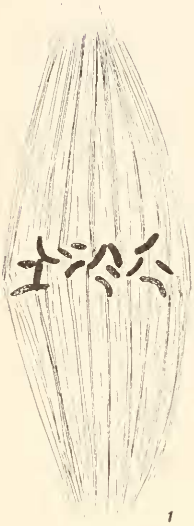
FIG. 6. F♀ × C♂; eggs radiated fifteen minutes; from one pole of anaphase of second cleavage. Same process carried farther.

FIG. 7. F♀ × C♂; both radiated three minutes; third cleavage division. A section through one pole of anaphase. All chromosomes are vesicles, showing no fusion, no condensation. The swelling process is still proceeding. The arrow points to the position of the sphere.

FIG. 8. F♀ × C♂; both radiated three minutes; fifth cleavage division, telophase. A somewhat oblique polar view. Nuclear division completed, but cytoplasmic not yet fully accomplished. Chromosomal vesicles further swollen, but quite distinct.

FIG. 9. Same material as Fig. 8. Telophase from one end of an incompletely divided fifth cleavage spindle. A so-called "reconstruction stage," with no sign of fusion of vesicles.

FIG. 10. Same material and stage. The aster at the right end is drawn in position from next section. The distinctness of the chromosomal vesicle at the side is particularly to be noted.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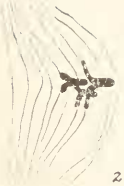
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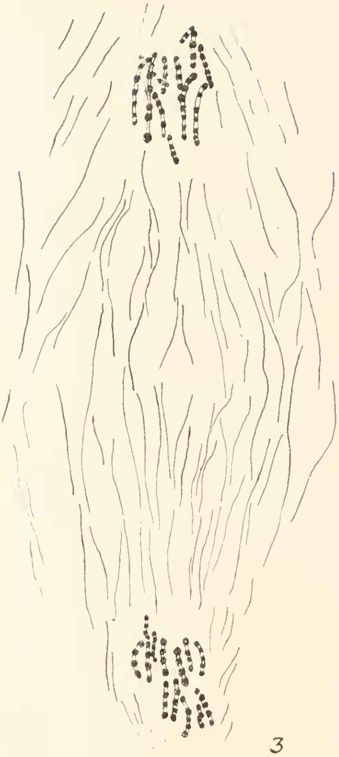
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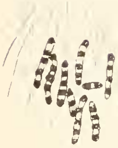
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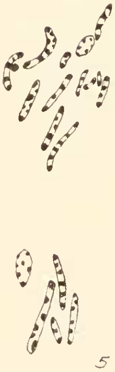
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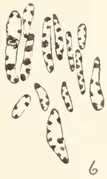
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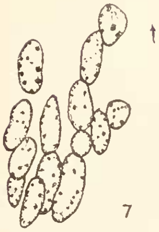
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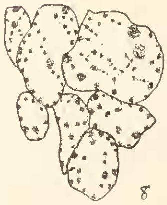
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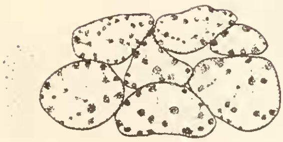
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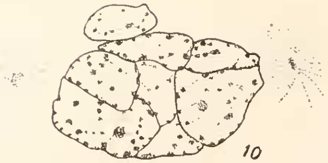
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EXPLANATION OF PLATE II.

FIG. 11. Same material and stage as preceding. Similar conditions shown. Rest of nucleus in next section.

FIG. 12. Same. It is to be noted that the division of the sphere has already taken place for the next mitosis.

FIG. 13. F♀ × C♂, normal material; condition of nucleus before the first cleavage division showing the distinct vesicles. Dotted line marks the area formerly occupied by this nucleus, shrinkage in preparation for ensuing mitosis having already occurred.

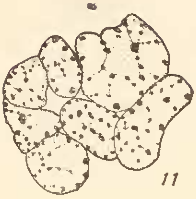
FIG. 14. F♀ × C♂; radiated for three minutes after fertilization. A nucleus from one-cell stage.

FIG. 15. F♀ × C♂; both radiated for three minutes before fertilization. Typical resting nucleus of second cleavage mitosis.

FIG. 16. F♀ × C♂; drawn with 6 objective and 4 ocular to show change in cell polarity. Blastula stage; upper cell is ectodermal; chromosomal vesicles shown in outline. Original axis was in line *AB*. With the shifting of the position of the spheres in telophase the axis of the upper cell came to line in line *CD* and that of lower in line *EF*.

FIG. 17. F♀ × C♂; eggs radiated for fifteen minutes before fertilization. Beginning of prophase of second division. Vesicles all distinct, showing a very definite polarity. Chromatin granules uniformly small and well distributed. This cannot be interpreted as merely a tangential section showing lobations, for the next section shows quite the same conditions.

FIG. 18. F♀ × F♂; from slide loaned by Miss Pinney as are Figs. 19, 20, 21 and 23. First cleavage similar to Fig. 17.



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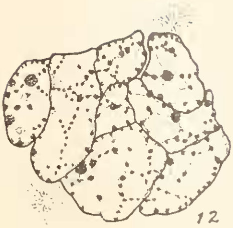

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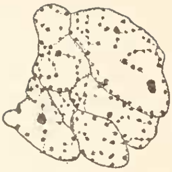

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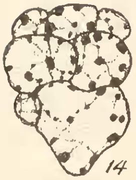

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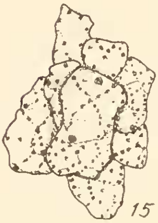

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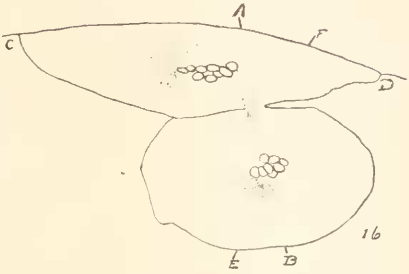

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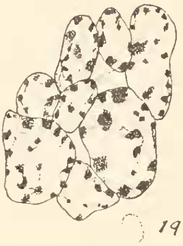

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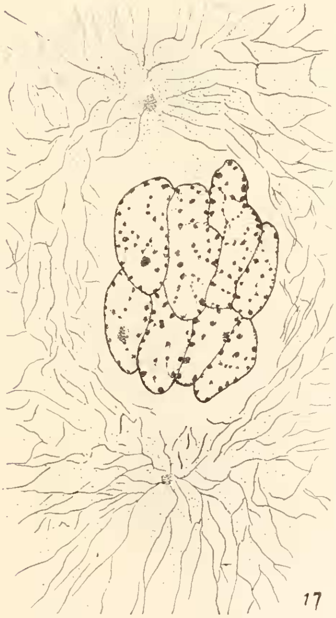

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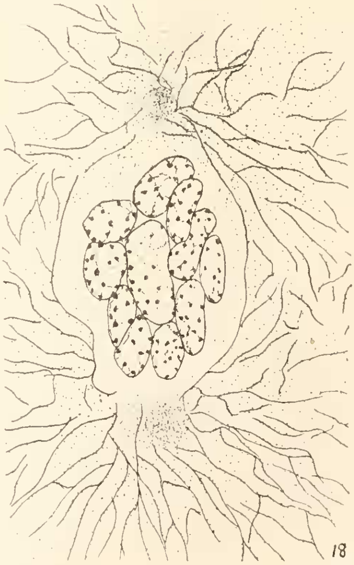

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EXPLANATION OF PLATE III.

FIG. 19. Pure *Fundulus* material. First step toward chromosome formation is shown in the condensation of chromatin granules on vesicle walls leaving the centers of the vesicles relatively empty.

FIG. 20. Pure *Fundulus* material. Further condensation and beginning of new chromosome as a row of granules, chromomeres, extending out into the center of the vesicle. Drawn as nearly as possible at one level.

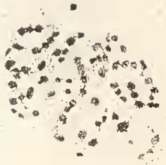
FIG. 21. All chromatin granules now formed into the new chromosome anlage. But the vesicular area can yet be distinguished. Only one level drawn.

FIG. 22. F♀ × F♂; radiated three minutes after fertilization. Prophase of third cleavage, showing distinctly old vesicular walls, and also new chromosomes already formed within. Certain of the vesicles show walls especially clearly. Only one level drawn.

FIG. 23. From slide belonging to Miss Pinney. Third cleavage. The only case observed showing the splitting of the chromosomes. The condition of spindle and asters suggest that this splitting may be precocious.

FIG. 24. F♀ × F♂; radiated three minutes after fertilization. Sixth cleavage prophase. oblique section. Traces only of the old vesicular walls are left while the astral rays are making their way into the chromosomes which are more condensed.

FIG. 25. F♀ × F♂; normal. Fourth cleavage, late prophase. As usual, only part of chromosomes are shown. Spindle formation completed and chromosomes are condensing and forming the equatorial plate.



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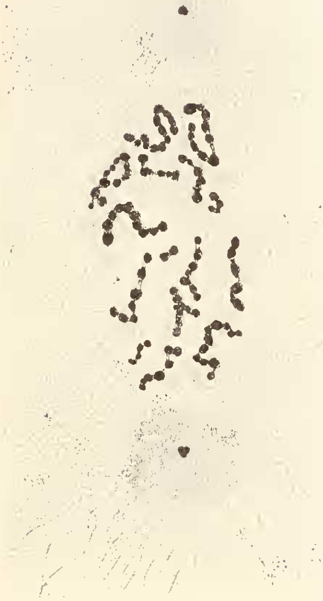

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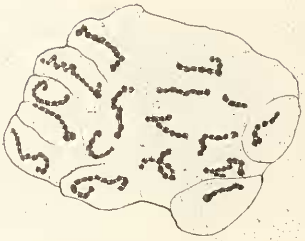

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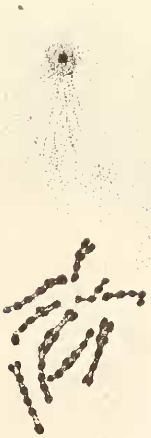

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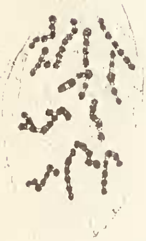

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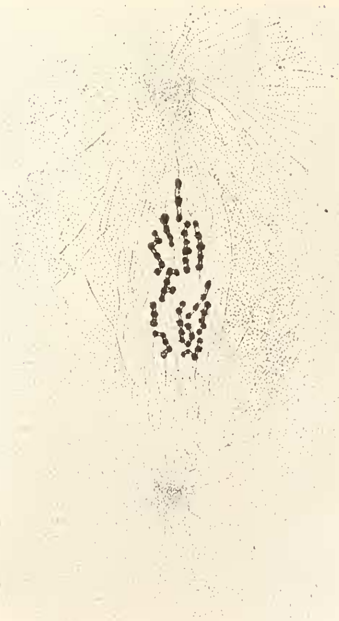

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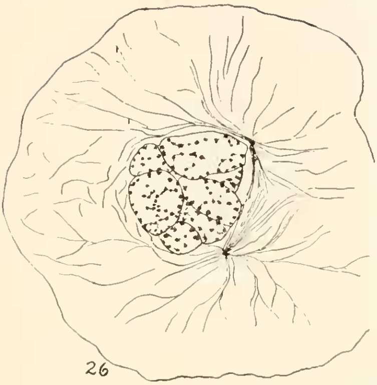
EXPLANATION OF PLATE IV.

Figs. 26 to 29 are from a grastrulation stage of F♀ × C♂; sperm radiated fifteen minutes before fertilization, by which the sperm chromatin was killed so that it took no further part in development.

Fig. 26 shows the central spindle forming across one side of the nucleus.

Fig. 27 is a later stage showing the central spindle half dividing the nucleus. Not female and male parts, for the sperm's contribution of chromatin was killed as shown in Fig. 29, an anaphase in which all chromosomes are of the *Fundulus* type, and in Fig. 30, which is a cross-section of such a nucleus showing the groove through which the central spindle passes.



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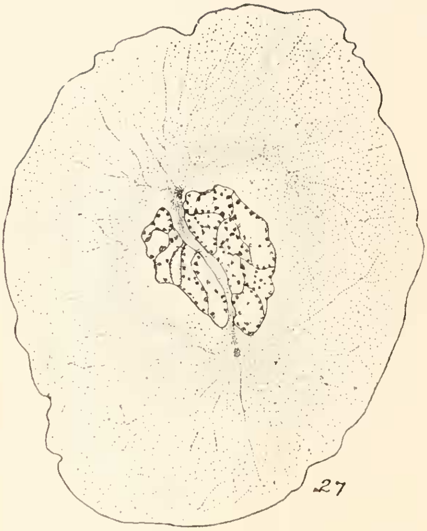

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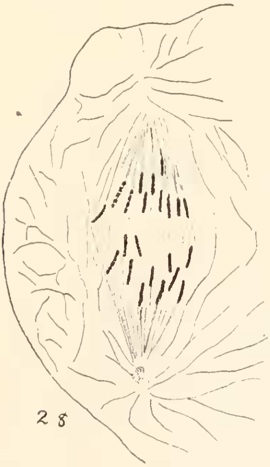

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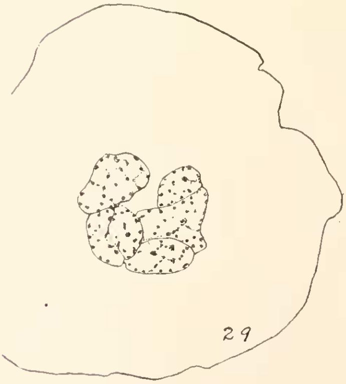

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Vol. XXXII. *May, 1917.* *No. 5.*

BIOLOGICAL BULLETIN

STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS.

I., DATA ON THE SEXES.[1]

J. T. PATTERSON.

I. Introduction.

According to Marchal ('04) and Silvestri ('06, '08) sex-determination in the polyembryonic chalcids is brought about in the same manner as in the bee. It is well known that the fertilized egg of the bee produces a female (queen or worker), while the unfertilized egg gives rise to a male (drone). Marchal and Silvestri believe that the fertilized egg of these parasitic hymenoptera produces a polyembryonic brood of females, while the unfertilized egg develops into a brood of males. This conclusion has been widely accepted, not only because it is in harmony with the Dzierzon theory of sex-determination in the bee, but also because several different investigators have observed that a polyembryonic brood usually does not contain individuals of both sexes. The occasional appearance of a mixed brood has been explained by the obvious and apparently logical assumption that a fertilized and an unfertilized egg had been deposited in a single egg of the host. The simultaneous development of two such eggs would naturally produce a mixed brood of parasites.

In a recent paper dealing with the development of the polyembryonic chalcid, *Copidosoma gelechiæ*, the writer ('15) called attention to the fact that it was difficult to explain the origin of certain mixed broods of this species on the assumption that two parasitic eggs carrying the opposite sex factors had been deposited in the egg of the host. It was suggested that of the nine complete, mixed broods listed, the origin of only five (broods 2, 3, 4, 6, 7, Table I.) could easily be explained in this obvious way. In each of the four remaining broods (broods 1, 5, 8, 9) the great preponderance of females seemed to indicate that such an explanation was not tenable. It was therefore suggested that these mixed broods could have arisen from a single fertilized egg, through a differential division of the two sex chromosomes at one of the early divisions of the egg. In that connection the following statement was made: "If *Copidosoma* conforms to the general scheme for sex-determination in insects, the females must have the 2 X chromosomes, and males the single X chromosome. Ordinarily, during the process of cleavage, all of the chromosomes in the fertilized egg divide equally, so that all of the nuclei entering into the formation of the embryos will carry the XX chromosomes, thus producing a brood of females. But if during the early development of the egg it should happen that the two X chromosomes in one or more cleavages should not divide but separate, one going to each pole of the spindle, each daughter nucleus would then receive a single X chromosome. If such nuclei later divide in the typical manner and give rise to embryos, such embryos would be males."[1]

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 130.

Different workers have from time to time offered a similar explanation to account for certain peculiarities in sex heredity, and especially to account for the origin of hermaphrodites and gynandromorphs. In doing so they have recognized the possibility that a qualitative division of the hereditary material for the sex factor may take place during cleavage. Their statements refer of course to the development of a monembryonic egg, in which the products of any somatic segregation must necessarily be distributed to different parts of the same embryo. In the case of a polyembryonic egg, a similar segregation, if occurring early enough in cleavage, would result in the production of two groups of embryos of different sexuality, and upon completing their development these two groups of embryos would produce a mixed brood.

It was this kind of evidence, although meager in amount,

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[1] *Loc. cit.*, p. 356.

that was discovered in studying the broods of *Copidosoma.* Only four out of a total of 162 broods gave evidence suggesting that a differential division might have occurred during the segmentation of the egg. It was therefore evident that if such differential divisions do occur in these parasitic insects, it must be studied in a species in which the polyembryonic brood is much larger than that of *Copidosoma.* For it is clear that the larger the brood the greater must be the number of cell divisions in that period of development at the close of which each blastomere becomes the progenitor of an embryo, and consequently. the greater the opportunity for a differential division to occur,

II. DATA ON SEXES OF PARACOPIDOSOMOPSIS.

At Austin such a species is found in *Paracopidosomopsis floridanus* Ashmead, a form very similar to the European chalcid, *Litomastix truncatellus,* upon which Silvestri worked. This little parasite lays its egg in the egg of the common cabbage looper, *Autographra brassicæ.* This species is very favorable for study, and can be reared in the laboratory. Upon consuming the contents of the *Autographra* larva, the larval parasites pupate within the skin of the host, forming the typical mummified carcass. During the last two seasons about two hundred carcasses have been collected from the field. The silken cocoons were removed from these, after which each was placed in a vial, which was closed with a loose plug of cotton. Under these conditions the parasites readily emerge.

TABLE I.

MIXED BROODS OF *Copidosoma.*

Brood.	No. of Individuals.	Females.	Males.
1	162	153	9
2	172	92	80
3	207	126	81
4	216	176	40
5	235	223	12
6	241	161	80
7	300	235	65
8	304	292	12
9	337	316	21

One hundred and twenty-nine of these broods, taken at random, have been studied in detail, with the result that a number of interesting facts concerning the sexes have been brought to light. Of the hundred and twenty-nine broods, three are pure female broods, sixteen pure male, and one hundred and ten mixed. The three female broods have 1,089, 1,306, and 1,859 individuals, respectively; while the sixteen male broods run as follows: 385, 432, 525, 578, 675, 699, 988, 1,003, 1,124, 1,277, 1,285, 1,288, 1,333, 1,354, 1,492, 1,588. This is an average of 1,001 individuals per brood.

It is the mixed broods which are of special interest. The number of individuals in these broods varies from 545 to 2,028, with an average of 1,246 individuals per brood. The percentage of males varies from 72.07 to .06. In Table II. are listed sixty broods, in which the sex of each individual has been carefully determined. These are arranged in the order of per cent. of males, from the highest to the lowest. Fifty additional broods are listed in Table III., in which the total number of individuals is given, and the per cent. of males as determined by a study of five hundred individuals in each brood. This method of studying the sexes does not give the exact number of males and females in a brood, but it yields a very close approximation to the true per cent. of males.

These data raise a number of interesting points, only a few of which can be considered here. One of the most striking features is the scarcity of pure broods. Of all the broods studied, only about twelve per cent. are male broods, and less than three per cent. female broods. Another equally striking feature is the great preponderence of females in the mixed broods. In only seven of the one hundred and ten such broods is the number of males in excess of the number of females (broods 1, 2, 3, 4, 5, 6, 61, Tables II., III.). In the remaining one hundred and three cases the females are in the majority.

Reference to Tables II. and III. will show that in over 58 per cent. of the broods less than ten per cent. of the individuals in any given brood are males, while in 35 per cent. of the cases there is less than three per cent. of males. The most striking cases are those listed toward the bottom of the tables, especially the last six or seven broods in each table. In each of the last

TABLE II.

MIXED BROODS OF *Paracopidosomopsis*.

Brood.	No. of Individuals.	Females.	Males.	Per Cent. of M a
1	974	272	702	72.07
2	1,289	400	889	68.97
3	1,237	482	755	61.02
4	636	294	342	53.78
5	828	385	443	53.50
6	545	272	273	50.09
7	1,162	595	565	48.71
8	848	529	319	37.62
9	1,512	1,009	503	33.27
10	1,847	1,287	560	30.32
11	839	586	253	30.15
12	943	701	242	25.66
13	998	746	252	25.25
14	1,706	1,353	353	20.69
15	628	509	119	18.95
16	662	542	120	18.12
17	1,022	878	144	14.09
18	1,364	1,195	169	12.39
19	1,797	1,584	213	11.85
20	1,041	923	118	11.35
21	1,477	1,357	120	8.12
22	1,115	1,033	82	7.35
23	1,307	1,211	96	7.35
24	1,303	1,199	104	7.21
25	1,196	1,122	74	6.19
26	803	754	49	6.10
27	1,432	1,352	80	5.59
28	868	822	46	5.30
29	1,423	1,349	74	5.20
30	1,050	996	54	5.14
31	1,883	1,791	92	4.89
32	1,259	1,203	56	4.45
33	1,457	1,394	63	4.32
34	855	828	27	3.16
35	1,046	1,015	31	2.96
36	1,124	1,091	33	2.94
37	1,000	972	28	2.80
38	757	739	18	2.38
39	847	827	20	2.36
40	1,301	1,271	30	2.31
41	1,209	1,183	26	2.15
42	1,260	1,233	27	2.14
43	1,682	1,647	35	2.08
44	1,592	1,559	33	2.07
45	1,258	1,232	26	2.07
46	692	679	13	1.88
47	1,220	1,200	20	1.64
48	1,115	1,097	18	1.61
49	1,036	1,021	15	1.46
50	1,798	1,774	24	1.35
51	1,075	1,061	14	1.30
52	1,475	1,456	19	1.29
53	1,349	1,336	13	.96
54	2,003	1,986	17	.85
55	1,017	1,010	7	.69

TABLE II.—*Continued.*

MIXED BROODS OF *Paracopidosomopsis.*

Brood.	No. of Individuals.	Females.	Males.	Per Cent of Males.
56	1,165	1,159	6	.52
57	1,143	1,141	2	.18
58	1,493	1,491	2	.13
59	912	911	1	.12
60	1,550	1,549	1	.06

two broods of Table II. only a single male was present. *In contrast with this condition, broods with a large number of males and a few females have so far not been found.* As stated above, in only seven cases out of a hundred and ten are males in excess of females, and even in these the smallest number of females is almost as much as 28 per cent. of the entire brood (brood 1). The fact that females are so frequently in excess of males in the broods is one of the most significant points brought to light in the study of these parasites.

TABLE III.

MIXED BROODS OF *Paracopidosomopsis.*

Per Cent. of Males Based on a Study of 500 Individuals.

Brood.	No. of Individuals.	Per Cent of Males.	Brood.	No. of Individuals.	Per Cent. of Males.
61	2,028	67.20	86	701	11.00
62	1,043	47.50	87	1,403	9.20
63	1,149	44.00	88	1,602	8.20
64	1,540	43.90	89	1,606	8.09
65	1,463	42.80	90	1,228	8.00
66	1,134	37.00	91	1,614	7.40
67	1,397	31.60	92	1,675	7.40
68	940	31.20	93	1,258	6.50
69	1,123	29.60	94	1,139	6.00
70	996	29.00	95	841	5.20
71	1,045	26.00	96	1,111	4.40
72	1,467	24.80	97	1,665	3.40
73	1,822	24.00	98	1,351	2.80
74	1,233	22.60	99	1,633	2.80
75	1,685	21.80	100	1,857	2.40
76	1,095	19.60	101	794	2.20
77	1,745	19.00	102	1,306	2.20
78	1,201	18.00	103	1,092	2.00
79	1,301	16.00	104	980	2.00
80	841	14.60	105	1,005	1.80
81	1,410	14.60	106	1,334	1.20
82	1,605	14.20	107	1,550	1.20
83	867	13.60	108	1,560	1.00
84	1,347	12.20	109	1,469	.60
85	1,167	11.80	110	1,227	.60

III. Suggested Explanations of Mixed Broods.

On the basis of the data here presented, how is one to explain the origin of mixed broods in *Paracopidosomopsis?* There are at least two possible explanations that may be suggested: (1) that a fertilized and an unfertilized egg had been deposited in the egg of the host; (2) that a fertilized egg had given rise to males as well as to females.

One finds no difficulty in offering the first of these suggested explanations to account for some of the mixed broods, especially for the larger broods in which males and females are found in about equal numbers. But if the same explanation is to be applied to all mixed broods, it would be necessary to assume that in the majority of cases the development of the unfertilized egg is interfered with by the presence of the developing fertilized egg. In the light of Lillie's ('16) recent discovery concerning the origin of the "free martin" in cattle, this assumption would not seem to be untenable. In the case of these insects it is conceivable that a fertilized egg might generate "hormones" which would inhibit the development of an unfertilized egg in such a way that only a few of the male embryos reach maturity.

One difficulty which at first seemed to stand in the way of the application of the hormone theory is the fact that the vast majority (about 85 per cent.) of all *Paracopidosomopsis* broods are mixed. It did not seem possible that eighty-five per cent· of the moth eggs, widely scattered as they are in the cabbage patch, would each be visited and parasitized by a fertilized and an unfertilized female insect. This raised the question as to whether the female lays more than a single egg at each oviposition, and if so, whether the fertilized female has the power to lay both kinds of eggs, as is the case of the queen bee. Silvestri states that the female of *Litomastix truncatellus* lays but a single egg at each oviposition. We have studied this question in *Paracopidosomopsis* and find that the female, whether fertilized or not, frequently deposits two eggs at one oviposition. In one experiment, a single unfertilized female made seventeen successive ovipositions in as many eggs. The time of oviposition varied from one minute and thirty seconds to three minutes, with an average of two minutes for each egg. An examination of these parasitized eggs in carefully teased preparations showed that the parasite had laid two eggs in eleven cases, and a single egg in six cases. The same experiment performed with fertilized females shows the same result. In a total of forty-two ovipositions made by three unfertilized and sixteen fertilized females, two eggs were laid in twenty-eight cases and one egg in fourteen cases.

While these observations show conclusively that two eggs are frequently deposited simultaneously by the insect, yet they do not settle the question as to whether the fertilized female can lay both kinds of eggs. However, a study of smear preparations and sections shows that in many cases both eggs are fertilized. For this, as well as for other reasons, the writer is of the opinion that any suggested explanation involving the idea that an unfertilized and a fertilized egg is laid in the egg of the host is inadequate to account for all of the mixed broods in *Paracopidosomopsis*.

This brings us to a consideration of the second suggestion, namely, that a fertilized egg may give rise to males and females. This result could be attained by the abnormal behavior of the sex chromosomes in one or more of the cleavage divisions. There are several different ways in which an abnormal behavior of the sex chromosomes might occur. Some of these possibilities are too improbable to receive consideration, but there are three or four that we shall mention. First, by *somatic disjunction*,[1] by which is meant the separation instead of division of the two sex chromosomes in a cleavage mitosis. Second, by *somatic non-disjunction*, by which is meant the failure of the two daughter chromosomes of one of the X's to separate after the division of both sex chromosomes had occurred. This would result in one daughter cell receiving three sex chromosomes, the other a single X chromosome. A third possibility is that only one X chromosome might divide, resulting in the formation of one cell with two X's and another with only one X. Finally, we may mention a fourth possibility, namely, that a division might occur in which all the sex chromosomes, whether divided or not, pass to one pole of the spindle. In this event one of the daughter cells would contain no sex chromosome. Let us consider further each of these possibilities.

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[1] This term is not entirely exact, because of the implication that the separating chromosomes had previously been united, which of course is not the case.

The mechanism of a disjunctional division was explained in connection with the study of *Copidosoma*, from which a quotation is given in the first part of this paper. It remains therefore to point out what would happen should such a differential division occur. If a disjunctional division should occur during cleavage, the percentage of males to females would be determined by the time at which the differential division occurs. For example, if disjunction took place at the first cleavage of the fertilized egg, each of the two daughter cells would receive a single X chromosome, and should no further disjunctions occur in subsequent divisions, a pure male brood would be produced. If on the same basis the disjunction did not occur until the second division, only fifty per cent. of the brood would be males; if at the third, twenty-five per cent.; if at the fourth, twelve and one half per cent., and so on decreasingly until the point is reached at which each cell becomes the progenitor of a single embryo. However, in any random lot of mixed broods these expected classes, based on per cent. of males would not be realized, because there are certain factors in development which would tend to modify the expected percentage of males in any given brood. Thus not all of the cells divide simultaneously in the early cleavages of the egg of these parasites. Some cells may fail to develop altogether. More than one disjunctional division may occur in the egg. Finally, some cells develop into the so-called assexual larvæ, which never reach maturity. In a random lot of broods we should therefore expect to find a continuous series, such as is shown in the tables.

Of the several possibilities suggested that of somatic non-disjunction would seem to be the most plausible. First, because it is in harmony with the known facts of cytology on cleavage mitosis, in that all of the chromosomes are supposed to divide; and second, because it is in accord with Bridges' ('16) work on non-disjunction in *Drosophila*. This investigator has recently suggested the possibility of somatic non-disjunction to account for gynandromorphs and mosaics in a monembryonic egg, such as that of *Drosophila*. He says: "If the same sort of primary non-disjunction which has been assumed to give rise to XX′X′ cells in an XX female, should take place at a cleavage stage, gynandromorphs and mosaics would result. One might expect at an early cleavage division, particularly the first, a relatively large number of X–XX′X′ divisions, for the greatly condensed chromosomes introduced by the sperm do not for some time attain the state or the appearance of those of the egg nucleus. If the paternal X of a female were slower than the maternal in preparing for division, it might lag upon the spindle so that both daughter X's would become included in the same cell. The portion of the fly which comes from the X cell should be male and should show the sex-linked characters of the mother. Such a process may be the explanation of the large number of lateral gynandromorphs of *Drosophila*. When the X–XX′X′ division occurs at a later cleavage stage we may have mosaics, as for example, a red female with a patch of white facets in the eye."[1]

In non-disjunction the per cent. of males in any brood would be determined, as in disjunction, by the time at which the division takes place. However, there would be this difference, that should the first cleavage be non-disjunctional, the expected percentage of males would be fifty instead of one hundred; should the second cleavage be non-disjunctional, twenty-five per cent. would be males instead of fifty, and so on, decreasingly throughout the series of cleavage divisions.

The third possibility mentioned above is in reality only a modified form of non-disjunction, and the percentage of males would be determined in accordance with the same law.

The fourth possibility suggested above is of interest because such a division would result in the production of blastomeres devoid of sex chromosomes. Cells which contain no X chromosomes may be responsible for the so-called asexual larvæ that develop from the egg of this species. We shall consider this point further in connection with a discussion of these peculiar larvæ.

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[1] *Loc. cit.*, p. 136.

IV. Experimental and Cytological Studies.

If any one of these forms of abnormal behavior of sex chromosomes does occur in the cleavage divisions of *Paracopidosomopsis*, it should be possible to detect it by experimental and cytological studies. We have undertaken both lines of investigation, and already sufficient progress has been made to clear up certain points.

The experimental work has been greatly interfered with by the ravages of the so-called wilt disease, to which the *Autographra* larvæ are extremely susceptible under laboratory conditions. In all of the experimental work the precaution has been taken to protect the moth eggs from being parasitized before beginning any experiment with them. This has been done by having the moth lay its eggs under a bell-jar from which all parasites were scrupulously excluded. Furthermore, the parasitized eggs and the young larvæ developing from them have been similarly protected. In all experiments the female parasite has been permitted to make but one oviposition in each moth egg.

The first set of experiments were undertaken with a view to determine whether the fertilized female parasite could lay eggs which produce males and females. In one experiment 23 moth eggs were parasitized by fertilized females. Sixteen larvæ hatched three days later. Of these two reached full growth. One of the two larvæ was not parasitized, and formed a pupa from which a moth later emerged. The other was transformed into a typical mummified carcass, from which a brood of parasites in due time emerged. The brood contained 2,096 individuals, of which 1,889 were females and 207 males. While these results are meager, nevertheless they indicate clearly that a fertilized female is capable of laying eggs from which individuals of both sexes develop. In this instance it is not possible to tell whether the mixed brood came from one egg or from two eggs, since there was no way of determining the number of eggs deposited by the parasite in the egg of the host at the time she made the oviposition.

In another set of experiments unfertilized females were used. In one experiment eighty moth eggs were parasitized, each by one oviposition by an unfertilized female. Sixty-six larvæ hatched. All but twenty-two of these larvæ were fixed at various stages of development, in order to secure material for the study of the unfertilized parasitic egg. Three of the twenty-two caterpillars eventually reach full or nearly full growth. Two of these showed signs of disease, and were killed, the parasitic larvæ being removed and fixed for cytological study. A study of sections of the larvæ from both caterpillars fail to show anything but male larvæ present. The third caterpillar formed a carcass, from which 1,842 males emerged. To this must be added 100 more individuals, which were removed from the carcass in the pupa stage and fixed for cytological purposes. The sections show that all the pupa are males. These results indicate that the unfertilized egg of *Paracopidosomopsis* produces males only.

A study of the spermatogenesis as revealed in these pupæ shows that males developed by parthenogenesis carry the haploid number of chromosomes, while a study of maturation of the eggs and fertilization shows that females, as would be expected, carry the diploid number. With these facts established, it will be possible to determine whether two types of males are produced, as may be shown by the following consideration. If *Paracopidosomopsis* conforms to the general scheme of sex determination in insects, the chromosomal formula for the fertilized egg may be represented by $N + 2X$, in which N represents the non-sex chromosomes, and that for the unfertilized egg by $\frac{N}{2} + X$. Now, should males also arise from the fertilized egg, their formula must be $N + X$. The presence of the double number of non-sex chromosomes in such individuals should not affect their sexuality, because in all forms in which parthenogenesis is absent, males are represented by this or a slightly modified formula. Modern genetic studies have shown that maleness or femaleness is determined by the number of X chromosomes present in the egg, and not by the number of non-sex chromosomes. Even in forms which have the Y chromosome, the presence or absence of the Y does not seem to affect the sex of the individuals.

V. The Asexual Larvæ.

Asexual larvæ were first described by Silvestri for *Litomastix*. According to this investigator, the egg of *Litomastix* produces a thousand or more sexual larvæ and a variable number of asexual larvæ, which are characterized by the absence of certain organs, and especially by the absence of the reproductive system. These larvæ die without undergoing metamorphosis. Exactly similar larvæ develop from the egg of *Paracopidosomopsis*. The details of their development have not as yet been studied, so that I cannot say whether they appear in every egg or not. These curious larvæ develop both in the fertilized and in the unfertilized egg, even though the host egg be rigidly protected from all other parasites. This shows that the asexual larvæ do not come from the egg of another parasitic species, as has been suggested as an explanation for their appearance among the larvæ of *Litomastix* (Wheeler, '10). An account of their development will be made the subject of a subsequent paper. Here, we are concerned with the question of the underlying cause of their production.

Silvestri suggests that these asexual larvæ may owe their asexuality to the absence of germ cells. It will be recalled that he described for the polyembryonic egg a so-called nucleolus (nucleolo), which lies at the larger or posterior end of the elongated, bottle-shaped egg. In the early cleavage stages of *Litomastix* the nucleolus becomes included in a single blastomere, resulting in the retardation of the subsequent divisions of this cell. The nucleolus later breaks down and becomes scattered evenly throughout the cytoplasm. Silvestri was unable to follow the history of this particular cell beyond two divisions, but he believes that it gives rise to all the germ cells of the sexual embryos. His conclusion has been strengthened by a subsequent study of the eggs of two monembryonic parasites. The nucleolus in the egg of these species becomes distributed to the germ cells alone, and thus serves as a "keimbahn-determinant" (Hegner).

According to Silvestri's suggestion the asexual larvæ of *Litomastix* arise from the polygerm, but fail to receive germ cells or descendants of the single blastomere which inherited the nucleolus. It may be that this suggestion of Silvestri offers the correct explanation of the origin of the asexual larvæ, but there are certain obstacles which stand in the way of its full acceptance. In the first place, it is difficult to conceive of any mechanism in the complex polygerm by which predestined germ cells could be nicely and exactly distributed to each of the several hundred sexual embryos which develop from the eggs of *Litomastix* and *Paracopidosomopsis*. In the second place, it is a well-known fact that in insects secondary sexual characters and also certain primary sexual characters, such as the organs of copulation and oviposition, are not dependent upon the presence of the gonads for their development. This conclusion is based upon trustworthy evidences obtained from castration experiments on young larval stages. Furthermore, in the light of modern genetic evidence, I should be inclined to believe that the asexual larvæ are sexless not because they have failed to inherit predestined germ cells, but because of their failure to inherit X chromosomes. If X-free blastomeres are formed during cleavage, such cells might become the progenitors of sexless larvæ, which in a way could be compared to the non-viable, OY zygote of *Drosophila*.

VI. Data on Sexes of Other Polyembryonic Insects.

With the exception of *Copidosoma gelechiæ* and *Paracopidosomopsis*, exact data on the sexes of polyembryonic insects are very few. The first observations recorded are those of Bugnion ('91), who studied twenty-one cases of *Ageniaspis* (*Encyrtus*) *fuscicollis* and found the following conditions: nine pure female broods; five pure male broods; three broods with males and females in about equal numbers; three broods with large majority of males; one brood with large majority of females. In commenting upon these data, Marchal ('04) points out that it is difficult to explain the four cases in which the large majority of individuals belong to one or the other sex. He believes, however, that such broods arise from two or more eggs. He further believes that should two eggs, one fertilized and the other not, be laid in the egg at slightly different times, the one deposited first might gain the upper hand in the matter of food, and thus prevent all but a few of the individuals arising from the second egg from reaching maturity.

Marchal examined sixteen broods of *Polygnotus minutus*, and found eight female broods, six male, and two mixed, one with three females and one male, the other with three females and three males.

The only other investigator to record data on the sexes of polyembryonic parasites is Silvestri. In one hundred and sixty broods of *Litomastix* he found sixty-three female, ninety-two male, and five mixed broods. He does not record the number of individuals for any of these broods.

I should like to point out in conclusion that there is very great need for exact data on the sexes of polyembryonic insects.

Austin, Texas,
January 17, 1917.

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ON THE PRODUCTION OF MONSTERS BY HYBRIDIZATION.

H. H. NEWMAN.

Of late there have appeared several papers that deal with the production of monsters in teleosts, especially in the common mud-minnow, *Fundulus heteroclitus*. The papers of Kellicott ('16) and Werber ('15 and '16) have been especially interesting because they deal with the same type of monster that I have been studying incidentally for more than ten years and that I have described and discussed in several different papers dealing with development and heredity in fish hybrids.

In 1908 I published the results of intercrossing *Fundulus heteroclitus* and *F. majalis* (Newman, '08). In the cross *F. heteroclitus* × *F. majalis* I called attention to the various types of monsters, noting especially those types in which the head and eyes developed and the body was relatively inhibited, and those in which head development was inhibited and body was relatively normal.

The wide range of variability in the results of crossing two individuals of different species was emphasized. A long list of conditions was given ranging from extreme subnormal eggs that failed to cleave, up to markedly supernormal individuals that are hardier and better equipped to live than individuals of either pure strain. In subsequent papers (Newman, '12 and '14) I called further attention to the graded series of teratomata resulting from one set of eggs fertilized by sperm from a single male.

In general it was shown that in hybrids between closely allied species (homogenic hybrids) the rate of development of a considerable percentage of individuals was accelerated, but that in all heterogenic hybrids there is a more or less marked retardation of developmental rate from the earliest cleavage stages on. Even in the case of homogenic hybrids, the retarded individuals are always abnormal.

During the summers of 1914 and 1916 a series of experiments was performed in order to test the theory that retardation is always correlated with abnormal development. In hybrid experiments in which *Fundulus heteroclitus* eggs are fertilized by the sperm of the mackerel there is noticeable in comparatively early cleavage stages a marked difference in number and uniformity of blastomeres in different eggs of the same batch. The different types of eggs were divided into three lots as follows:

A. Those that were most advanced and exhibited the largest number and greatest regularity of cells.

B. Those in which the cells were fewer and less uniform.

C. Those in which the cells were relatively fewest and of various sizes.

The development of these three lots was watched from day to day with the result that all of those in A reached a fairly advanced condition before abnormalities appeared and abnormalities concerned chiefly the eyes and heart; those in B showed all sorts of monsters including cyclopeans, eyeless types, and those with reduced bodies; those in C produced solely apical parts without bodies, amorphous masses of tissue or at best isolated organs such as eyes and hearts.

The converse of this experiment was tried using the eggs of *F. heteroclitus* and the sperm of the closely related *F. diaphanus*. In this experiment there was very little acceleration noticeable during the cleavage stages, but during gastrulation it was a simple matter to separate out three classes A, B, and C as follows:

A. Those in which the germ ring was distinctly more than half way round the yolk.

B. Those in which the germ ring was approximately half way round the yolk. These were about like the control.

C. Those in which the germ ring was distinctly less than half way round the yolk.

The eggs in lot A nearly all hatched on the average distinctly earlier than the control (pure *F. heteroclitus*), and were distinctly more active, grew faster and lived longer than the control. Those in lot B showed a wide range of variability, some hatching as early as the control, others hatching later, and still others failing to hatch. Various common types of abnormality occurred but none of the most extreme types were noted. Those in lot C showed the most pronounced abnormalities. Only a few hatched and these were rather feeble. The remainder were monsters belonging to a wide range of types, many showing merely some defect in the circulation (string-hearts, enlarged pericardia, no blood, etc.), others being defective in the head parts, especially eyes, and others being defective chiefly in the posterior parts.

These two experiments were repeated many times and gave substantially identical results each time. There can be no question therefore that *there is a very close correlation between the rate of development and the degree of normality of the embryos.*

When in homogenic crosses a slight increase in rate of development is instituted the result is a supernormal F_1 hybrid type, in which the difference is largely physiological, consisting of greater activity and greater viability. When in the heterogenic crosses there results a more or less pronounced retardation in developmental rate (sometimes clearly seen in the early cleavage stages) a subnormal type or monster is the result, which differs from the normal not only physiologically in being non-viable but morphologically in being defective in one or more parts.

Both supernormal and subnormal types appear then to be correlated with an alteration in the normal rate of development.

It is quite evident from numerous experiments (Newman, '15) that the degree of retardation resulting from varous heterogenic hybridizations is not a factor of the distance of the cross, for some species of the same genus cross with poor success, and some species of different orders of teleosts cross so as to produce swimming larvae. This argues against the theory that foreign sperm introduces toxins, since the protoplasm of distantly related forms ought to be more toxic than that of near relatives.

About all we can say then is that *in introducing a foreign sperm into an egg we either accelerate or retard the developmental rate of the egg.* In proportion as the rate is accelerated we get a supernormal result, and in proportion as the rate is retarded, a subnormal result.

That there is nothing specific about the effect of foreign sperm upon the developmental rate of the egg is further shown by the fact that the same series of subnormal types (monsters) are found in a great variety of different crosses, and also by the fact that these same abnormal types may be produced by purely physical or by chemical agents.

Other Methods of Producing Monsters.

As long ago as 1907, Stockard, by the use of solutions of magnesium chlorid, was able to produce, among other abnormal conditions, a considerable percentage of cyclopic and allied ophthalmic terata. Although little was said about abnormal conditions other than those associated with the particular terata mentioned, enough data was given to show that many if not all of the other well-known conditions occurred.

In 1909 and 1910 Stockard contributed further papers concerning the development of teratomata, showing that the same results could be obtained with alcohol and other anæsthetics. He called particular attention to ophthalmic terata, making it clear that the eye anlages appear to be especially susceptible to anæsthetics, and explained cyclopia and similar conditions as "the result of an anæsthetic action during the early developmental stages." Later, when McClendon ('12) obtained a similar series of eye defects by the use of substances that are non-anæsthetic in action, Stockard took the more general view that deleterious chemical substances merely lower the vitality of certain sensitive anlages and cause arrested development of important structures. Stockard implies that the results are primarily due to a lowering of developmental rate. The paired eyes for example are believed to arise from an unpaired median anlage which normally undergoes a sort of twinning process producing two eyes. Under the influence of deleterious chemicals "the median anlage does not widen or spread laterally but is arrested in its primary median condition." Hence the unpaired cyclopean eye develops.

Recently Werber (1915, 1916) in three papers and especially in his latest one (1916) re-attacked the problem from a somewhat novel point of view. Starting in with the assumption that the development of monsters in mammals is caused by "pathologic parental metabolism" he decided to test his hypotheses with fish embryos. He consequently subjected the embryo of the fish *Fundulus* to solutions of various metabolic by-products, characteristic of normal and pathologic mammals, such as urea, butyric acid, lactic acid, acetone, sodium glycocholate, and ammonium hydroxide. Conclusive results were obtained only with butyric acid and acetone, especially the latter. The paper is especially valuable for its comprehensive record of terata, their classification, and the numerous figures of monsters of every sort. All of the results are similar to those obtained in hybridization experiments, as is evidenced by the fact that the illustrations would serve as well for a paper on hybrid teratomata as for the paper in which they appear. All of Stockard's teratomata are also duplicated exactly.

It is especially to be noted that there are two markedly different types of monsters: (*a*) those in which the head parts alone are inhibited, and especially the eyes and heart, and (*b*) those in which the head parts develop fairly normally and the posterior parts are inhibited. Extremes of (*a*) are seen when only the eyes or heart are effected, and of (*b*) when eyes or heart develop alone to the exclusion of other parts of the body. These results appear at first sight quite paradoxical, but the explanation will be clear from what follows.

Werber appears to realize the importance of Child's theory of the "axial gradient" in organisms as an aid in understanding the morphogenesis of monsters. He points out that the anterior end, or "apical end" is most susceptible to agents that inhibit development, and hence we have a logical basis for the frequency of ophthalmic terata. He sees in Child's work however no explanation of the occurrence of heads with reduced body and of isolated eyes, hearts, etc.

Werber's idea of the morphogenesis of monsters differs somewhat from that of Stockard in being strongly morphological. According to him any chemical substance, whether anæsthetic, toxic, or what not, so long as it is injurious to life, checks the process of cell metabolism and cell division and causes the affected cells to *disintegrate*. This "*differential blastolysis*" accounts to Werber's mind for the suppression of certain anlages, especially of those at the apical end of the embryo; but it fails to account for the development of isolated eyes and hearts in other embryos of the same series, which are treated exactly as those were in which the eyes were suppressed or the heart failed to develop.

It will be seen that both Stockard and Werber believe that abnormal development is the result of toxic or deleterious substances introduced into the egg from without. It has been shown however that identical results can be obtained by purely physical methods.

In a very recent paper Kellicott ('16) has reported the *production* of every known type of teratomata in *Fundulus* by merely subjecting very young embryos to low temperatures. Eggs placed in the refrigerator a few minutes after insemination very nearly stopped development and some apparently underwent regressive changes. When returned to normal temperatures these eggs developed nearly every type of abnormality. The most frequent terata were those of the eyes and of the heart. A list of terata would be a mere repetition of those obtained by other methods.

By way of a theory of the genesis of monsters Kellicott proposes what he calls a "disorganizational hypothesis," namely "that the cause of abnormal and monstrous development, is to be found in a disturbance of the normal organization of the ovum, as expressed by the unusual character and distributions of the differentiated materials of the egg protoplasm." It should be noted in connection with these low temperature experiments that the first result is a very pronounced lowering of developmental rate, amounting in some cases almost to a cessation of development, accompanied by certain regressive changes. There is also in all cases a permanent retardation even after the embryos are returned to normal temperatures. It seems then that *the primary effect of low temperature is the lowering of metabolic rate.*

It will now readily be seen that the whole series of teratomata in fish eggs may be produced in three entirely different ways, the results being the same in each:

1. By the use of chemicals whose action is such as to lower the rate of metabolism.

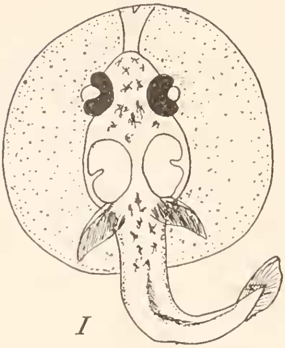
2. By means of low temperatures, which a~~e~~ffect~~s~~ directly the rate of metabolism.

3. By means of heterogenic hybridization, which always effects a lowering in the rate of development.

All monsters then seem to be the result of a lowering of developmental rate.

Let us now return to a consideration of hybrid monsters and ask ourselves the question: *how does the foreign sperm lower the rate of development?* Various views are held as to the way in which the foreign sperm retards development.

Moenkhaus holds that the foreign sperm exercises a poisonous or toxic effect in proportion to the distance of the cross. Loeb holds that the results are due to the inability of the hybrid to digest the yolk with sufficient rapidity. I have expressed the view that there is an incompatibility between the nuclear elements of the two different species that results in abnormal chromosome arrangements and irregular distributions of the latter to various cells. Undoubtedly also the cleavage mechanism works less perfectly when chromosomes of very different size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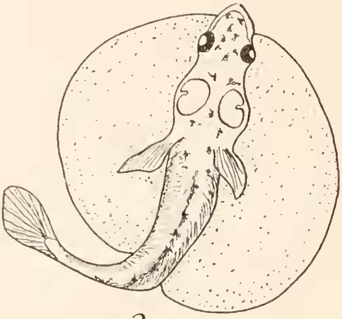
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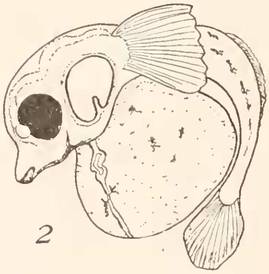
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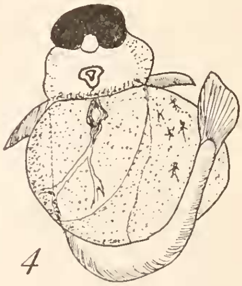
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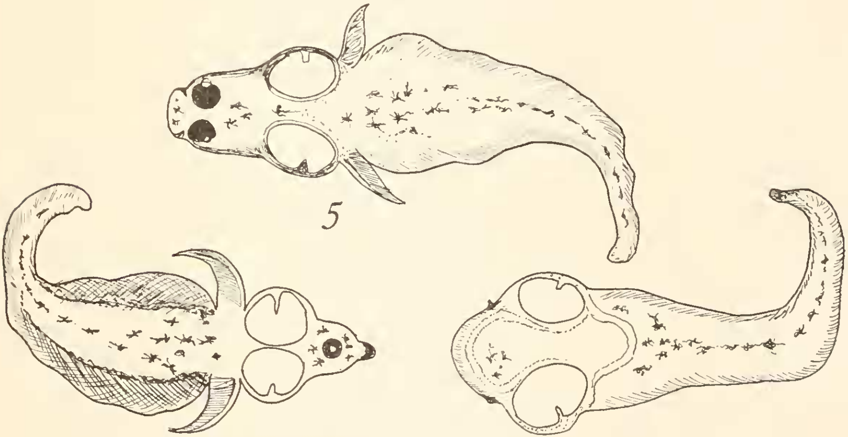
and form are mixed together in a single cell, than when they are all of the same kind. Whatever the primary cause of abnormal development may be, the most obvious early effect is a lowering of developmental rate and it is on this point that the similarity exists among all of the experimental results heretofore discussed.

Since we are dealing primarily with rates of metabolism, we shall doubtless find, as Werber has suggested, that the morphogenesis of monsters in teleosts is simply a special case of form regulation similar to those recently described and discussed by Child ('15 and '16) for sea-urchins, in which he demonstrates the effectiveness of the axial metabolic gradients as dynamic factors in the development of various types of monsters. He has been able to "control and modify development by means of the differential action of external factors on different regions of these gradients."



It is my belief that an application of the principles enunciated by Child serve to rationalize the results of heterogenic hybridization as well as those produced by chemicals and by low temperatures, and since Werber merely suggested the possibility of explaining ophthalmic anomalies by this theory and failed to apply it in any far-reaching way to the other various types of monster he was dealing with, it seems worth while to give the theory a thorough trial in this field.

The Axial Gradient Theory and the Morphogenesis of Monsters Resulting from Heterogenic Hybridization.

The axial gradient in vertebrates is essentially a quantitative one depending on the rate of metabolic activity. The rate along the apico-basal axis is highest at the anterior or apical end and lowest at the posterior or basal end. Paralleling the axial metabolic gradient there exists a susceptibility gradient, in which the apical end is most susceptible to agents that lower the vitality or retard the rate of metabolism. Similarly the median dorsal region is the most susceptible point of the bilateral or mesiolateral axis and the lateral part the least susceptible. Certain points that are both apical and median will be the most susceptible of the whole system. The eyes are such structures developed from a primitive mesio-dorsal-apical anlage, and are therefore the most susceptible of all parts of the body.

In animals of the high grade of complexity exhibited by vertebrates there are to be distinguished several semi-independent axiate systems. Undoubtedly the central nervous system and its derivatives constitutes an axiate system by itself. Similarly the circulatory system is axiate with the heart the apical point and the veins and arteries basal. The skeleton with the fin systems are also semi-independent axiate systems, with the anterior parts more susceptible than the basal ones. This is brought out in recovery cases in which the pectoral fins recover while the pelvic do not.

Whenever young fish embryos are subjected to any of the types of inhibiting or retarding agents that have been previously discussed we are able to classify the resulting abnormal forms into categories (modifying somewhat the usage of Child): (*a*) Forms in which there has occurred an inhibition of anterior and of median dorsal regions; (*b*) Forms in which these regions have become acclimated or have recovered from an initial inhibition. Curiously enough in chemical experiments those parts that are most susceptible to inhibiting agents of higher concentrations are able most completely to acclimate themselves in lower concentrations or to recover when the inhibiting agent is removed or its severity lessened. Let us consider these two categories of teratomata in some detail.

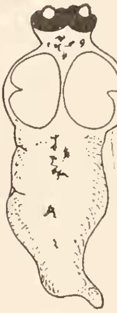
(A) Forms Resulting from Differential Inhibition.

In fish monsters, whether they are the result of chemical, physical, or biological (hybridization) inhibitors, the most prevalent abnormalities are associated with the eyes and with the heart. The teratomata resulting from all three types of inhibiting agents are strikingly similar, so I shall from now on confine my description and discussion to monsters produced by heterogenic hybridization. In any heterogenic cross the least abnormal forms are always those that have something slightly abnormal or sub-normal about the eyes or the heart. We must conclude then that the primordia of these two structures are peculiarly susceptible to agents that retard metabolic rate.

Ophthalmic Terata.—Eyes in the least extreme cases may be either a little too small, a little too close together, of unequal size, or asymmetrical in position. In the most extreme cases the eyes may be wanting, or very poorly differentiated; the eyes may be fused more or less completely (various grades of cyclopia) or there may be a single unilateral eye. Every intergrade between the least extreme and the most extreme ophthalmic terata may be readily found in almost any hybrid experiment. Why in a single batch of eggs there should be so highly diversified a result is not known, but it must be due to physiological (age, maturity, etc.) differences in the individual eggs or sperm, or a combination of both.

In general we may conclude that suppressed eyes, either bilateral or unilateral, are the result of inhibition primarily of the apical end of the apico-basal gradient, while eyes too close together or fused in the median line (cyclopic terata) are the result primarily of inhibition of the median portion of the mesio-lateral axis.

Cardiac Terata.—All grades of heart abnormalities occur in heterogenic crosses. The less extreme cases are those in which the heart development is belated relatively to the other bodily regions, or in which there may be a failure of the whole cardiac mechanism to become inclosed within the body cavity. In such cases the heart remains outside the body and there is usually a much enlarged pericardium. In the most extreme cases the heart never develops, or becomes a mere pulsating strip of muscular tissue running from the body to the yolk of the egg across the pericardium. These "string-hearts" are among the commonest terata to be found in heterogenic hybrids. We have no choice but to conclude that, although the heart is not very close to the apical end, and is ventral rather than dorsal in position, that it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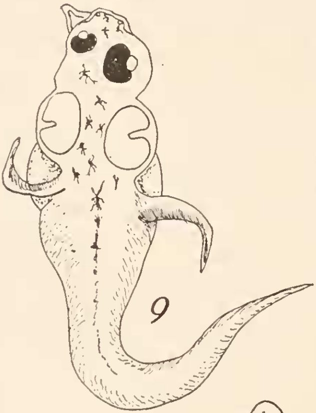
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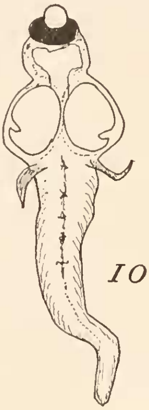
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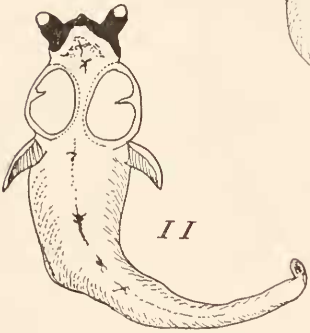
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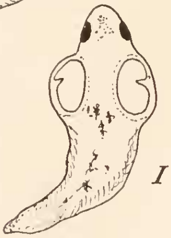
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is an organ of relatively high rate of metabolism and shows extreme susceptibility to retarding agents. It is undoubtedly true that the heart and blood vessels form an axiate system of their own, which is somewhat independent of general somatic axes. The attached or arterial end (ventricles) of the heart is the apical point of this axis and is the part most susceptible to retarding agents. When this end of the heart is inhibited the whole circulatory system will be rendered functionless, though the basal parts may be fairly well developed. When the apical parts are only slightly abnormal the basal parts may be fully normal, but when the apical parts show extreme abnormality the basal parts are usually abnormal but less so than the apical.

(B) Forms Resulting from Differential Acclimation or Recovery.

Of somewhat less frequent occurrence than the above described types of fish hybrid teratomata are those in which the apical and mesial parts have become acclimated to (or adjusted to) the materials brought in by the foreign sperm. The less extreme types are those in which the head is relatively large and wide as compared with the trunk and tail. Next comes a whole series of types in which first the tail and then the trunk are inhibited more or less completely, and even heads without trunks but with beating hearts are common. The most extreme cases are those in which isolated eyes or isolated hearts grow upon an otherwise undifferentiated blastoderm (Fig. 14). I have found several examples of isolated eyes that could be distinguished only by the presence of a lens and of pigmented retinal cells. Also isolated hearts may be reduced to small pulsating "drums" of tissue in which there is no axiate elongation. According to the axial gradient theory not only are the apical points most susceptible to inhibitors, but under certain circumstances, such as long exposure to low concentrations, they have the highest capacity for recovery. That is why eyes and hearts, the apical parts of the two axiate systems, show the highest capacity for recovery and are sometimes the only parts that do recover after long and general inhibition. A very common type of teratomata is one in which the whole body is decidedly abnormal, inhibited no doubt at an early stage, but in which recovery of the apical structures has occurred to various degrees, so that we have forms in which the only highly differentiated parts are apical parts. Such embryos are shown in Figs. 13 and 14.

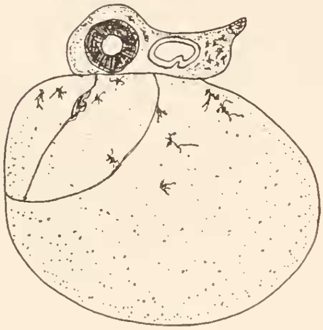
Child ('16) distinguishes between forms resulting from differential acclimation, forms resulting from differential recovery, and forms resulting from differential inhibition with general recovery. While I believe that the forms resulting from hybridization are identical to those produced by chemical inhibition, I am not able to say in any particular case whether a form is one or the other of these three types. My judgment is that the isolated eyes and hearts are forms resulting from differential recovery and that the forms with comparatively well developed head and small or rudimentary body are the result of differential acclimation. After all, the results of acclimation and recovery even in controlled chemical experiments are so nearly identical that, were it not for the differences in experimental method used, they would be indistinguishable.

On the whole then we can distinguish clearly only the two types of monsters:

(*a*) Forms with inhibited apical parts and relatively well developed basal parts—the results of differential inhibition.

(*b*) Forms with inhibited basal parts and relatively well developed apical parts—the results of differential acclimation or recovery.

None of the forms of fish hybrids with which I am acquainted fail to fall into one of these two categories and to receive a logical explanation on the basis of the axial gradient theory. It is my belief that this theory offers the best explanation of teratological development in fish and in all other axiate forms, including man.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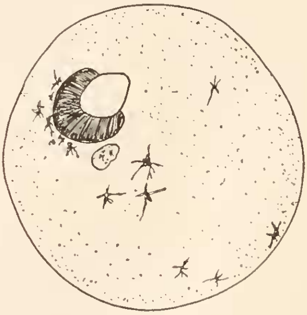
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As a concrete illustration of the application of the theory to a single experiment, I shall offer the details of an intensively studied hybridization experiment, the one referred to earlier in the paper in which the three lots of hybrid eggs were separated on the basis of the rate of early cleavage into three lots A, B and C. This experiment dealt with hybrids of *Fundulus heteroclitus* ♀ × *Scomber scombrus* (mackerel) ♂.

Figs. 1–4 show types of teratomata in lot A. These embryos are frequently almost normal up to an advanced stage and a very few hatch as perfectly normal larvæ. A considerable percentage of embryos develop a circulation but have comparatively small eyes as in Fig. 1. Others have a proboscis and the eyes both look forward as in Fig. 2. Still others have a very narrow head with small eyes and the heart is a string-heart as in Fig. 3. Others, finally, are cyclopic forms in which the mesio-lateral development of the head has been more seriously inhibited than the apico-basal (as in Fig. 4).

The largest and most varied assemblage of monsters is found in Class B in which the rate of cleavage was intermediate between the lowest and highest rate. Types of embryos of this group are shown in Figs. 5–12, which are camera lucida drawings made when embryos were two weeks old. In all of these the whole body from head to tail is abnormal (compare with the embryos of A in which the tail is nearly normal). Ophthalmic terata are most common here. Fig. 5 is microphthalmic and a little asymmetrical and the body and tail are fairly normal; pectoral fins are also developed and the otic vesicles are only slightly enlarged. Fig. 9 has decidedly asymmetrical eyes, one very small; the jaws, pectoral fins, etc., are also asymmetrical, but the tail is fairly well developed. Fig. 11 shows a peculiar type of stalked-eye, which is rather common; body is only slightly abnormal; pectoral fins normal. Figs. 6 and 10 are two types of cyclopic monster with reduced and shortened tail, but with enlarged otic vesicles and good pectoral fins. Fig. 8 is a synophthalmic type with enlarged otic vesicles and much reduced body. Fig. 12 is a common form with much reduced eyes, well developed otic vesicles and small body. Fig. 7 is an eyeless type in which the otic vesicles seem to have usurped the place of the eyes in the body; the body is fairly well developed. All of the types here shown had the heart beating. All of these forms I look upon as products of acclimation after an early partial inhibition. They were not as much retarded as those in lot C. I look upon the enlargement of the otic vesicle as the result of the suppression or partial suppression of the dominant region anterior to it. The otic region now is to some extent physiologically isolated and thus released to grow to a larger size than normal.

I shall show only two types out of a very large number found in lot C in which the slowing of the cleavage rate was most pronounced. Fig. 13 shows a common type which is practically a head without a body. The little up-turned stump is all there is of the body; the heart is still beating; the eyes are in an advanced stage of differentiation. Fig. 14 shows a case of solitary or isolated eye. No other tissues are differentiated. I look upon these as cases of recovery of the apical regions after rather severe early inhibition. There is no hard and fast line to be drawn between the three classes of monsters shown.

It may be said in closing that each type of hybrid cross differs from others in the particular array of monsters displayed. In some there is never cyclopia, but always microphthalmia. In some the extreme recovery types, like that shown in Fig. 13, are much the most common. In many respects the *F. heteroclitus* ♀ × mackerel ♂ cross is the best adapted for the study of the morphogeny of teratomata because of the very wide range of forms produced.

In conclusion I wish to express my appreciation of the far-reaching applications to morphogenetic phenomena of the axial gradient idea as worked out by Child. Few generalizations known in biological science serve to rationalize so wide a range of developmental phenomena.

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RESULTS OF CROSSING EUSCHISTUS VARIOLARIUS AND EUSCHISTUS ICTERICUS WITH REFERENCE TO THE INHERITANCE OF TWO EXCLUSIVELY MALE CHARACTERS.

KATHARINE FOOT AND E. C. STROBELL.

The two exclusively male characters selected for study in this cross are first, the black or dark brown spot on the male genital segment of *Euschistus variolarius* and, second, the intromittent organ, which differs markedly in length in the two species.

In two recent papers[1] we described in detail the transmission of the above-mentioned exclusively male characters in a cross between *Euschistus variolarius* and *E. servus* and the results from the study of the transmission of these two characters in the cross of *E. variolarius* with *E. ictericus* are merely a confirmation of the results obtained from the cross of *E. variolarius* with *E. servus.* The results from the latter cross were published first because they were more satisfactory, for the reason that the spot which is so pronounced on the male genital segment of *variolarius* is entirely absent from the male segment of *E. servus*, whereas it is not entirely absent from the genital segment of *E. ictericus.* Although *ictericus* is described as having no spot on the genital segment, a faint spot is frequently present. This is not easily determined until the segment is pulled out and closely examined, but the spot, if present, can then be clearly seen, though it is not nearly so pronounced as that of *variolarius.* (Compare photos 61 and 62.) Although this spot is not a constant feature in *ictericus,* it is present often enough to make it impossible to determine how much of the inheritance of the spot in the hybrids is due to the female *variolarius* and therefore the results from this cross are less decisive, on this point, than the results from the cross with *servus*.

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[1] "Results of Crossing *Euschistus variolarius* and *Euschistus servus* with Reference to the Inheritance of an Exclusively Male Character," *Journ. Linn. Soc. London*, Zoöl., Vol. XXXII, 1914.

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The intromittent organ, however, is a chitinous spiral which can be dissected out and accurately measured, making it possible to determine the exact size relations in the hybrid. We shall, therefore, first describe the results obtained from a study of the inheritance of this organ and shall then more briefly state the less definite results obtained from a study of the genital spot.

Dr. Eltringham's discovery of the difference in the length of the intromittent organ between the two species *E. variolarius* and *E. servus* (Foot and Strobell, '15) led us to expect a difference in the length of this organ between the two species *E. variolarius* and *E. ictericus*. The three species do, in fact, differ greatly in this character. At a magnification of twenty diameters, the mean length of the intromittent organ of *E. servus* is 166.41 mm. of *E. variolarius*, 96.70 mm. and of *E. ictericus*, 60.64 mm.

Our cross-breeding experiments with *variolarius* by *ictericus* differ in important details from our experiments with *E. variolarius* by *E. servus*. In the latter we succeeded in raising to maturity, eleven males and 16 females of the F_1 generation, and from these we were able to raise a large number of the F_2 generation (204 males and 249 females). In the *variolarius* × *ictericus* cross, on the contrary, we succeeded in raising only one specimen of the F_1 generation (a female). We were successful, however, in crossing this F_1 female with a pure *ictericus* male and we raised from this back-cross 76 males and 73 females. These results are a gratifying supplement to our cross-breeding experiments with *variolarius* and *servus*, for in the latter, a back-cross was commenced so late in the season that we were able to secure only 18 males and 8 females.

Details of Crossing Euschistus variolarius × Euschistus ictericus.—The three female *variolarius* used for this cross were hatched in our laboratory, and immediately after the fifth moult were caged with three male *ictericus* which were also hatched and reared in the laboratory. The parents of the three females were two of ten specimens of *variolarius* collected at White

Plains, N. Y., April 16, 1911, by Mr. de la Torre Bueno. These bugs were placed in a cage in our laboratory and, four days later, one pair mated, and while mating were transferred to a separate cage, in which they were kept during the entire breeding season, until the female died.[1]

Three of the female offspring of this pair were used for the cross-breeding experiments with *ictericus*. They reached the winged stage on the following dates: July 3, one female reared from the group of 4 eggs deposited May 22; July 15, two females reared from the group of 29 eggs deposited June 9. The three male *ictericus* that were caged with these three *variolarius* females were reared from eggs deposited in our laboratory May 22 and June 2. These eggs were deposited in a cage containing ten *E. ictericus* specimens that had been collected a few days before by Dr. Knab in a swamp near Washington, D. C. One of the males from these eggs reached the winged stage July 7 and two, July 15 and the three were caged with the three abovementioned *variolarius* females.

August 12 a pair of these bugs mated and while mating were transferred to a separate cage (cage 5). The record of this pair shows that they mated 6¼ hours[2] and did not mate again while they were under observation (from August 12 to November 24, 1911) when the female died. On August 15, 7 eggs were deposited, none of which developed. On August 18, 4 eggs were deposited one of which hatched and was raised to maturity—this being the F_1 female which we mated the following spring to a pure *ictericus* male. On August 25, 23 eggs were deposited, none of which developed. (See photo 63 for the male of this pair.)

A second pair mated August 19 and this pair also was isolated (cage 13). A group of 8 eggs was deposited by this second pair August 22 and a group of 18 eggs on August 25 but none of these 26 eggs developed.

[1] The record of the breeding period of this pair of *variolarius* was published in our report of the results of crossing *E. variolarius* × *E. servus*. This record is entered as "cage 2, 1911." Foot and Strobell, '14, *Journ. Linn. Soc. London*, Zoöl., Vol. XXXII, p. 362.

[2] The length of time for each mating can be given only approximately as observations were made not oftener that three or four times each hour, during the day and three or four times during the night.

The last pair of bugs of the original three pairs never mated, although they were kept under observation until October 11. They were then added to a cage containing eight specimens of the same cross (4♀ and 4♂) which we had caged to carry through the winter.

The record of the first pair shows that from August 15 to August 25, 34 eggs were deposited and that only one of these developed. If we add to these the above-mentioned 26 eggs from the second pair of this cross, we find that out of 60 *variolarius* eggs, that were presumably fertilized by *ictericus*, only one developed and hatched.

Comparing these results with those of the cross between *variolarius* × *servus*, we find that in the latter a very much larger proportion of eggs developed, for out of a total of 120 eggs, 37 developed and 32 of these hatched.

This contrast between the results of the two crosses is greatly increased if we add the number of eggs deposited by the *variolarius-ictericus* cross of the summer of 1912. As stated above we caged through the winter of 1911–12 five female *variolarius* and five male *ictericus*. All of these were reared in the laboratory from the same stock as those of the 1911 pairs. Only one of these mated the next season (cage 12, 1912). Of the 53 eggs which were deposited between June 11 and July 15 not one developed. We believe we might have had better success with wild males; but we failed in our efforts to get them in the spring of 1912. In summing the results we find that 113 eggs in all were deposited by the three *variolarius* females that were crossed with *ictericus* and that only one of these 113 eggs hatched; the remaining 112 did not show even the initial stages of development. This one egg that developed was deposited August 18 and was not hatched until August 28, as the weather was unseasonably cold. The nymph (a female) reached the winged stage September 26. We succeeded in keeping this hybrid in good condition through the winter of 1911–12, and the following June she mated with a pure *ictericus*. The record of this pair is as follows:

RECORD OF CAGE 3, 1912.[1]

F_1 Hybrid ♀ (from *E. variolarius* × *E. ictericus*) and pure *E. ictericus* ♂—one pair.

June

15 3.45 P. M. mating. Continued to mate 17 hours.

21 20 eggs (in two groups) 15 survived first moult.

24 8 eggs. 7 hatched.

28 6 eggs. 5 hatched.

29 13 eggs. 12 hatched.

33 reared to winged stage (16♂ and 17♀).

When some of the nymphs from the above four sets of eggs reached the third or fourth moult, they were transferred to a large cage and therefore some specimens from each of these four sets were combined and thus the individuality of each set was lost.

July

5 12 eggs. 10 hatched. 9 reared to winged stage (3♂ and 6♀).

6 14 eggs. All hatched. 13 reared to winged stage (5♂ and 8♀).

8 7 eggs (on wire top of cage } 12 hatched. 9 reared to winged stage (2♂
9 6 eggs (on wire top of cage } and 7♀).

12 12 eggs. (5 of them on wire top of cage) 10 hatched. 9 reared to winged stage (5♂ and 4♀).

16 8 eggs. All hatched. 7 reared to winged stage. 5♂ and 2♀.

21 5 A. M. 6 eggs (on wire top of cage). All hatched. All reared to winged stage. (4♂ and 2♀.)

21 5 A. M. mating. Continued to mate 21 hours.

24 18 eggs. All hatched. 16 reared to winged stage. (8♂ and 8♀.)

30 22 eggs (in 5 groups). 21 hatched. 18 reared to winged stage. (10♂ and 8♀.)

August

6 5 eggs } All hatched. 9 reared to winged stage. (5♂ and 4♀).
9 6 eggs }

13 14 eggs (in 4 groups). 12 hatched. 8 reared to winged stage. (5♂ and 3♀.)

17 12 eggs (in 2 groups). 11 hatched. Later added to nymphs from eggs deposited August 28.

22 3.30 P. M. mating. Continued to mate 48 hours.

28 6 eggs (scattered on wire top of cage). 5 hatched. Later added these to nymphs from eggs deposited August 17. 12 reared to winged stage. (8♂ and 4♀.)

30 Killed both the male and female. Both were inert and apparently nearly dying. (The female has had only four legs since July 12.) Preserved in glycerine (tube 33). See photo 64 for the male.

All the females of this pair and 20 males are preserved as pinned specimens; the rest of the males are preserved in glycerine. See photos 65–69 for twenty-five specimens.

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[1] The data of this record are taken from our notes from which we have selected only the essential items, omitting such details as the dates of hatching, the dates when the five moults occured and the number of nymphs that survived each moult. The eggs hatch from five to seven days after deposition, this variation depending largely on the temperature .

A comparison of the above record with those of pure *variolarius* shows that in this back-cross the relation between mating and deposition of eggs is not normal, for, as a rule, eggs are deposited not oftener than once or twice between two matings, whereas in this cross, eggs were deposited eleven times between the first two matings and six times between the last two matings. The number of eggs, however, that were deposited between June 21 and August 28 is above our average for pure *variolarius* and a normal proportion of these eggs developed. 195 eggs were deposited and 177 of these hatched. 149 were reared to the winged stage. (76♂ and 73♀.) All of these females are preserved as pinned specimens and the males are preserved either as pinned specimens or in glycerine.

Intromittent Organ.—This organ is a chitinous spiral easily dissected from the genital segment of the males (photos 1 to 60).

In accordance with Dr. Eltringham's suggestion, the entire male segment was first softened in warm caustic potash, until the intromittent organ was sufficiently pliable to be dissected out without breaking.

Only a single organ was mounted on a slide, and the slide was so indexed that the insect from which the organ was taken could be identified and therefore, the relation of the intromittent organ to any other character could be satisfactorily studied in one and the same insect. This was possible in the case of the genital spot, for the genital segment of all the specimens which were preserved in glycerine had been photographed before the segment was removed for dissection.

Each intromittent organ being mounted in balsam, it was photographed at a magnification of 20 diameters, and all measurements were made from these photographs. The actual length, therefore, of each organ is one-twentieth of the length recorded in this paper.

Measurements from such photographs of the intromittent organs of *E. variolarius*, *E. ictericus* and of the offspring from the back-cross are given in the following three tables.

Table I. gives the lengths of the intromittent organs of 62 specimens of *E. variolarius*. This table is quoted from our report of results from the cross between *E. variolarius* and *E. servus*. (Foot and Strobell, '15, see footnote, p. 322.)

Table II. gives the lengths of the intromittent organs from 32 specimens of *E. icterius*.

Table III. gives the lengths of the intromittent organs of 71 offspring from the back-cross of a pure *icterius* male with the one F_1 female we succeeded in raising from crossing *E. variolarius* ♀ by *E. icterius* ♂.

As the intromittent organ of *E. icterius* is much shorter than that of either *E. variolarius* or *E. servus*, it was much less difficult to get an accurate measurement of the length of the organ in *icterius* and this was true also of the offspring from the

Table I.

Lengths of intromittent organs from 62 specimens of *Euschistus variolarius* × 20 diams.

85.5 mm.	1
87 mm.	1
88 mm.	1
89 mm.	1
90 mm.	1
91 mm.	1
92 mm.	2
93 mm.	4
93.5 mm.	1
94 mm.	6
94.5 mm.	2
95 mm.	1
95.5 mm.	2
96 mm.	7
96.5 mm.	1
97 mm.	1
97.5 mm.	1
98 mm.	6
98.5 mm.	1
99 mm.	2
100 mm.	7
100.5 mm.	1
101 mm.	4
102 mm.	3
104 mm.	1
104.5 mm.	2
106 mm.	1
5,996 mm.	62

Mean length = 96.70 mm.

Table II.

Lengths of intromittent organs from 32 specimens of *Euschistus icterius* × 20 diams.

54.5 mm.	1
55 mm.	1
58 mm.	3
59 mm.	1
60 mm.	8
60.5 mm.	3
61 mm.	2
62 mm.	6
62.5 mm.	2
63 mm.	3
64 mm.	1
64.5 mm.	1
1,940.5 mm.	32

Mean length = 60.64 mm.

back-cross. We used, however, the same method as for the longer and more complicated coils of *servus*, *i. e.*, photographing each intromittent organ at a magnification of 20 diameters, and measuring the photographs with a small pair of architect's dividers. (For details see Plates I.–III., p. 337.)

TABLE III.

Lengths of intromittent organs from 71 specimens of offspring from back-cross *i. e.*, F_1 ♀ (from *E. variolarius* ♀ × *E. ictericus* ♂) by *E. ictericus* ♂ × 20 diams.

60 mm.	2
62 mm.	2
62.5 mm.	1
63.5 mm.	1
64 mm.	4
66 mm.	8
66.5 mm.	3
67 mm.	1
68 mm.	7
68.5 mm.	1
69 mm.	7
70 mm.	10
70.5 mm.	2
71 mm.	3
72 mm.	7
72.5 mm.	2
73 mm.	1
73.5 mm.	1
74 mm.	3
74.5 mm.	1
75 mm.	1
76 mm.	2
76.5 mm.	1
4,897.5 mm.	71

Mean length = 68.97 mm.

Genital Spot.—A study of the inheritance of the genital spot in the offspring from the back-cross of *variolarius-ictericus* is of interest merely as furnishing additional data in support of the results given by the crosses of *variolarius* by *servus*. These results, however, are by no means as definite as those from the intromittent organ, for the dimensions of the genital spot can be only relatively stated, they cannot be accurately measured as in the case of the length of the intromittent organ. The influence of each parent in the transmission of the genital spot in the hybrids can, therefore, be ascertained only approximately and the case is further complicated in the *ictericus* cross by the fact that in *E. ictericus*, unlike *E. servus*, a genital spot is not wholly absent.

Photo 61 is of seven male specimens of *E. variolarius* showing on each genital segment, the brown or black spot characteristic of the *variolarius* male, and photo 62 is of seven specimens of *E. ictericus* showing the faint indication of a genital spot which is characteristic of nearly all *E. ictericus* males. As stated above, the spot, when present, is so indistinct that in most cases it is easily overlooked unless the segment is pulled out and closely examined, and this is perhaps why this species is described as having no spot on the genital segment. Van Duzee's description is as follows:

"*Euschistus ictericus* Linn.

"Found in the Northern States and Canada across the whole width of the continent. It is generally to be found on sedges in swampy spots or along the borders of streams or other bodies of water. It may be distinguished from the foregoing [*E. variolarius*] by the calloused ruga connecting the humeri which are more produced than in *variolarius*, and the genital segment of the male wants the black spot found in that species."[1]

Although the presence of a faint spot in *ictericus* is an embarrassing factor in comparing the results of the *variolarius-ictericus* cross with those of the *variolarius-servus* cross, it by no means obscures the fact that the evidence from the two crosses is in complete harmony.

The *servus* crosses demonstrated that the genital spot was transmitted through the female of the first cross, and this is supported by the *ictericus* back-cross. The inheritance from the *variolarius* female of the first cross can be appreciated by comparing the faint spot of the pure *ictericus* specimens of photo 62 with the spot in some of the back-cross offspring, for example the first specimen of photo 65, the second specimen of photo 66, the third and last specimens of photo 69. In these the spot is almost as strong as that of the pure *variolarius* males and much stronger than any of the genital spots of the pure *ictericus* males.

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[1] Our *Euschistus ictericus* specimens were identified by J. R. de la Torre Bueno.

The *variolarius-servus* crosses further demonstrated that the genital spot was transmitted directly from the male and this is also supported by the *variolarius-ictericus* back-cross. The *ictericus* inheritance is shown in all the photographed specimens in which the spot is quite as insignificant as the faintest spot in the pure *ictericus* specimens.

Although the harmony in results with those of the *servus* experiments is evident, the factor of an indefinite spot in *ictericus* makes a detailed comparison less satisfactory and for this reason we shall discuss the results from the two back-crosses in relation to the intromittent organ rather than the genital spot. These two exclusively male characters (as we demonstrated by the *servus* crosses) give exactly the same evidence, in every detail, in their bearing on current theories under discussion.

Results and Discussion.

The results obtained from this back-cross, F_1 ♀ (from *variolarius* × *ictericus*) by *ictericus*, are so nearly a repetition of those obtained from the back-cross of F_1 ♀ (from *variolarius* × *servus*) by *variolarius*, that they would seem scarcely to merit separate publication: but for the fact that the results obtained from the few specimens secured from the latter cross were of small scientific value, until supported by more data, and such data (from *ictericus*) were not in shape for publication when the results of the *servus* cross went to press.

One point of interest in the back-cross of F_1 ♀ by *variolarius* was that the offspring did not show a Mendelian type of inheritance, but that the length of the intromittent organ appeared to be a quantitative response to the proportion of inheritance from the two species.

In attempting to measure the strength of the inheritance in this cross, we found that the effect on the length of the organ corresponded almost exactly with the relative amount of *variolarius* to *servus* in the offspring. This was 3 *variolarius* to 1 *servus*, as *variolarius* was first crossed with *servus* and a male *variolarius* used for the back-cross with the F_1 female. In our report of this cross we stated the results as follows:

"If we measure the amount of the *variolarius* inheritance in this back-cross, we find an astonishing agreement between the theoretical expectation and the actual result. The relative amount of *variolarius* to *servus* in the offspring of this back-cross is 3 to 1, and we should expect, therefore, the mean length of the intromittent organ of *servus* to be reduced by 75 per cent. of the difference between the mean lengths in *variolarius* and *servus*. This difference is 69.71 mm., 75 per cent. of this being 52.28 mm. Deducting this from the mean length of the organ of *servus* (166.41 mm.) would leave 114.13 mm. as the mean length of the organ of the offspring from this back-cross. The mean length is in fact 113.47 mm., this being only 0.66 mm. less than the calculated expectation."[1]

If our results had followed a Mendelian type of inheritance, assuming the *servus* length of organ to be dominant, we should expect the mean length of the intromittent organ to be approximately 131.33 mm. instead of being the above simple expression of the proportion of inheritance from the two species.

This result would seem scarcely to merit serious consideration, as it was based on the inheritance from only 18 males; but from the back-cross reported in the present paper (*variolarius-ictericus*) we succeeded in getting 76 males and these repeat the evidence given by the 18 males of the first experiment.[2]

The two experiments are entirely similar except that in the first experiment the back-cross was made with a male of the same species as the female of the first cross (*variolarius*) and in the second experiment the back-cross was made with a male of the same species as the male of the first cross (*ictericus*).

If we look for an agreement in results from the two experiments, we should expect the mean length of the intromittent organ of the 71 males from the second back-cross to show a like quantitative response in inheritance to that in the case of the 18 males of the first experiment.

In this second back-cross we have 3 parts *ictericus* to 1 *variolarius*, and we should expect the mean length of the intromittent organ of *variolarius* to be reduced by 75 per cent. of the difference between the mean lengths in *ictericus* and *variolarius.* This difference is 36.06 mm., 75 per cent. of this being 27.04 mm. Deducting this from the mean length of the organ of *variolarius* (96.70 mm.) would leave 69.66 mm. as the mean length of the organ from this back-cross. The mean length is in fact 68.97 mm., this being 0.69 mm. less than the calculated expectation. In both back-crosses the slightly stronger inheritance is in favor of the shorter type of intromittent organ and from the species giving 75 per cent. of the inheritance.

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[1] Foot and Strobell, '15, "Results of Crossing Two Hemipterous Species with Reference to the Inheritance of two Exclusively Male Characters," *Journ. Linn. Soc. London*, Zoöl., Vol. XXXII., p. 474, 1915.

[2] The intromittent organs of 71 of these 76 males were measured. See page 329.

The 71 males from this back-cross further support the results of the first experiment as follows:

The back-cross (*variolarius-servus* by *variolarius*) showed that the length of the intromittent organ was transmitted directly from the male and also indirectly through the female, and that therefore the so-called male- and female-producing spermatozoa do not differ functionally in their transmission of a character so exclusively male as the intromittent organ.

Its direct transmission by the male was proved by the decrease in the mean length of the organ of the offspring from the back-cross as compared with that of the F_2 hybrids and it is further shown by the F_1 generation of the *variolarius-servus* crosses. The facts are as follows:

Mean length of the organ—

In the *variolarius* species	96.70 mm.
In the *servus* species	166.41 mm.
In the F_1 hybrids	124.9 mm.
In the F_2 hybrids	124.42 mm.
In the offspring from the back-cross	113.47 mm.

The *increase* in the mean length of the organ in the F_1 hybrids as compared with that of the *variolarius* species proves the direct transmission from the male parent, and the *decrease* in the mean length of the organ in the offspring from the back-cross as compared with that of the F_1 and F_2 generations shows again the direct influence of the male parent, the *servus* male of the first cross increasing the length towards that of the *servus* species and the *variolarius* male of the back-cross reducing it towards the *variolarius* species.

These results are supported by the *variolarius-ictericus* back-cross though the evidence of the *direct* influence of the male parent might be questioned in this cross, as we did not succeed in securing any F_1 or F_2 males, and this would leave open the question whether the *ictericus* inheritance in these offspring was due in part to direct inheritance from the male of the back-cross or wholly due to indirect inheritance through the F_1 female. In the *servus* experiments, on the contrary, the direct influence of the male in both the first cross and the back-cross is beyond question.

Evidence of the transmission of this exclusively male character through the female is given in both back-crosses, but in the *variolarius* by *servus* experiments it is most conclusively shown by the mean length of the intromittent organ of the F_1 generation, where the inheritance from the pure *variolarius* female of the first cross is clearly in evidence.

In the *ictericus* experiment, the inheritance from the pure *variolarius* female of the first cross is shown in the increased mean length of the intromittent organ of the offspring of the back-cross as compared to that of the pure *ictericus* species. The facts are as follows:

Mean length of the organ—

In the *variolarius* species	96.70 mm.
In the *ictericus* species	60.64 mm.
In the offspring from the back-cross	68.97 mm.

A further point for consideration in the two back-crosses is the fact that in the first (*variolarius-servus*) the 25 per cent. inheritance is from the *male* of the first cross (*servus*) and in the second (*variolarius-ictericus*) the 25 per cent. inheritance is from the *female* of the first cross (*variolarius*). As in the one case, this one quarter inheritance was received directly from the male (*servus*) and in the other case indirectly through the female (*variolarius*), and as these have so nearly the same value in the offspring from both back-crosses, we would seem to have the strongest evidence that the so-called male- and female-producing spermatozoa do not differ quantitatively in their transmission even of a character so exclusively male as the intromittent organ.

In the *variolarius-servus* by *variolarius* back-cross we have direct evidence that this exclusively male character—the length of the intromittent organ—is transmitted by the so-called female-producing spermatozoa, for the *servus* inheritance is from the F_1 female, which must have developed from an egg fertilized by a female-producing spermatozoön of *servus*.[1]

Thus the evidence that not only the so-called male-producing spermatozoa but also the so-called female-producing spermatozoa can transmit even such an exclusively male character as the intromittent organ, and the fact that these so-called sex-determining spermatozoa do not differ functionally in the transmission of such a character certainly justifies much scepticism as to that sex-determination hypothesis which is based on the assumption of male- and female-producing spermatozoa.

In the reports of our *variolarius* by *servus* experiments we have repeatedly discussed the bearing of the results on the interesting and ingenious hypotheses which attempt to locate Mendelian factors not only in the chromosomes as such, but in definite chromosomes and in definite areas of definite chromosomes, and we have pointed out that the evidence from our results was in direct contradiction to such hypotheses and, further, that the evidence, as stated above, was against the hypothesis that assigned the rôle of sex-determination to the "sex-chromosomes" of the so-called male- and female-producing spermatozoa. As the evidence bearing on these questions is simply repeated in this *variolarius* by *ictericus* cross, we would (to avoid repetition) refer our readers to a recent paper[2] where we have briefly discussed the evidence in relation to these questions.

We feel almost like apologizing for our persistent criticism of these ingenious chromosome hypotheses so brilliantly launched by Morgan and his pupils[3] and so vigorously advocated by Wilson.[4]

Our scepticism was perhaps primarily due to fourteen years' patient study of the chromosomes of *Allolobophora fœtida*, these years of investigation of one form forcing us to the conviction that the chromosomes share a variability that is admittedly a characteristic of other organs of the cell and a characteristic of all organic structures. Such convictions as to the morphological nature of the chromosomes made it impossible to have faith in any hypotheses that were based on the type of individuality of the chromosomes demanded by the theories in question.

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[1] For the sake of the argument we must assume, for the moment, the existence of male- and female-producing spermatozoa.

[2] Foot and Strobell, *Journ. Linn. Soc.*, London, 1915, pp. 475–486.

[3] A complete list of the literature by Morgan and his pupils is given in their recent volume, "Mechanism of Mendelian Heredity," Morgan, Sturtevant, Muller and Bridges. Henry Holt & Company, 1915.

[4] A forceful presentation and endorsement of these hypotheses was recently given by Wilson in his Croonian Lecture. *Proceedings Royal Soc.*, ser. B, Vol. LXXXVIII., No. B 603.

In a recent paper, Trow[1] gives an able and searching criticism of one of these hypotheses and he has the sympathy of all sceptics when he voices the pressing need of an accurate knowledge of the chromosomes, especially of the maturation divisions where alone can be found the cytological support essential to a foundation for such a superstructure of imaginary architecture. This need is further voiced by Bateson[2] in his report on a recent volume by Morgan and his pupils.[3] Of the maturation prophases he says: "That twisting takes place in many types, especially Amphibia, is clear; but neither the figures reproduced from Janssens nor the originals from which they are taken—still less the very fragmentary observations of both Stevens and Metz from *Drosophila*—provide more than a slender support for this most critical step in the argument. It is to be hoped that the authors will before long tell us exactly upon what evidence they are here relying."

It is well known by cytologists that, with our present methods of technique, an accurate knowledge of the maturation divisions of *Drosophila* is impossible and one is led to suspect that this ignorance as to the chromosomes leaves the imagination a freedom that perhaps would not be possible if curbed by more hard facts.

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[1] "A Criticism of the Hypothesis of Linkage and Crossing Over," *Journ. Genetics*, Cambridge, England, Vol. 5, No. 4, 1916.

[2] *Science*, N. S., Vol. XLIV., No. 1137, p. 536.

[3] "The Mechanism of Mendelian Heredity."

EXPLANATION OF PLATES I.–III.

We photographed each intromittent organ at a magnification of 20 diameters and prints on matte paper were made from these negatives. Then each subject was accurately measured with a small pair of architects' dividers fitted with no. 9 needle points, and set at 2 mm. The measurements were made on the matte prints so that each division of 20 mm. could be indicated by a pencil mark and numbered. The measurements were taken from the distal end of the intromittent organ to the point where the thick part of the coil enters the gland. The coil is most easily dissected off at this point (*e. g.*, photos 3, 5, 6, 8, etc.) but in those cases where part of the canal within the gland has been preserved (*e. g.*, photos 1 and 2) the point from which the measurement was made may be readily determined, for the part within the gland is transparent and quickly tapers to a finer canal which apparently extends through the entire gland. The transparency of that portion of the canal within the gland is much clearer in the preparations than in the photographs, for when the chitin has a yellow tinge it appears black and opaque in the photograph.

In many specimens we have left a small piece of the gland at the point where the intromittent organ enters (*e. g.*, photos 7, 9, 10, 12, 14, 19, etc.).

PLATE I.

PHOTO 1. Intromittent organ of *Euschistus variolarius*—the father of the *E. variolarius* female that was fertilized by the pure *E. ictericus* male.

Length of intromittent organ 96 mm.

PHOTOS 2, 3 and 4. Intromittent organs of three brothers of the *E. variolarius* female that was fertilized by the pure *E. ictericus* male. From this cross we raised the F1 female which was back-crossed with a pure *E. ictericus* male and produced the offspring described in this paper.

Length of the intromittent organ of photo 2, 100 mm.—of photo 3, 94 mm.—of photo 4, 99 mm.

PHOTOS 5 to 8. Typical specimens of the intromittent organ of *Euschistus variolarius*.

Length of the intromittent organ of photo 5, 93 mm.—of photo 6, 93.5 mm.—of photo 7, 97.5 mm.—of photo 8, 98 mm.

PHOTO 9. Intromittent organ of the *Euschistus ictericus* of the original cross (cage 5, p. 324). This male *ictericus* was raised in our laboratory and fertilized the *Euschistus variolarius* female from which we obtained the F1 female used for our back-cross experiment.

Length of the intromittent organ 60 mm.

PHOTO 10. Intromittent organ of the *Euschistus ictericus* that fertilized the F1 female . . . (the father of all the offspring from our back-cross experiment). (See record, p. 326.)

Length of the intromittent organ 63 mm.

PHOTOS 11, 12 and 13. Intromittent organs of three of the original stock of *Euschistus ictericus* that we received from Washington, D. C., May 18, 1911.

Length of the intromittent organ of photo 11, 62 mm.—of photo 12, 60 mm.—of photo 13, 62.5 mm.

PHOTO 14. Intromittent organ of an *E. ictericus* male that was raised in our laboratory in 1911, kept through the winter, and in 1912 mated with a pure *E. variolarius* female. (See account of cage 12, p. 325.)

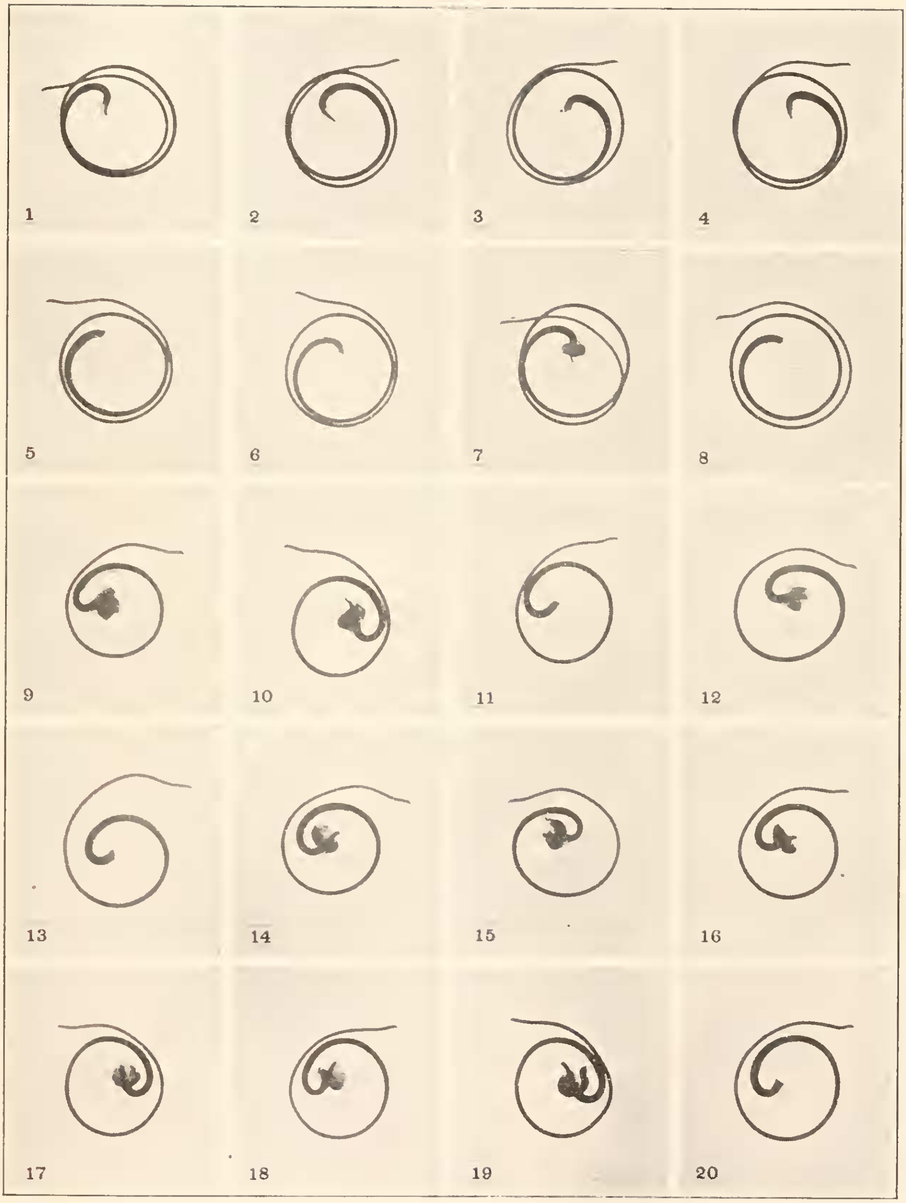
Length of the intromittent organ 60 mm.

PHOTO 15. Intromittent organ from one of the brothers of the *E. ictericus* that was used for the original cross with *E. variolarius*. These were raised in our laboratory in 1911.

Length of the intromittent organ 54.5 mm.

PHOTOS 16 to 20. Intromittent organs from five typical *E. ictericus* males that were raised in our laboratory during the summer of 1911.

Length of the intromittent organ of photo 16, 58 mm.—of photo 17, 59 mm.—of photo 18, 60 mm.—of photo 19, 61 mm.—of photo 20, 64.5 mm.



K.F. & E.C.S. photo.

INTROMITTENT ORGANS of E. VARIOLARIUS & E. ICTERICUS

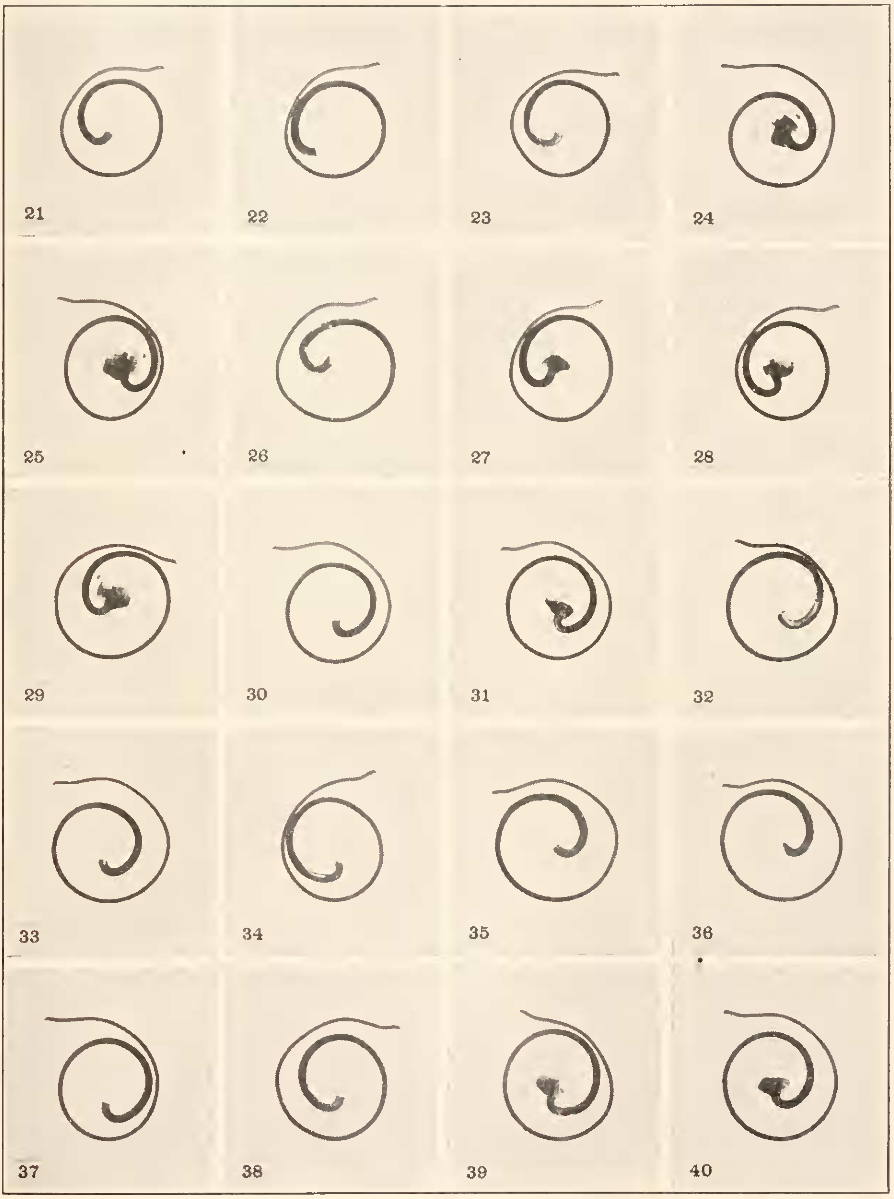
PLATES II AND III (PHOTOS 21–60).

The photographs of these two plates show typical intromittent organs from 40 of the 76 males which we succeeded in rearing from the back-cross with *E. ictericus* male and the F1 female obtained from a cross with *E. variolarius* ♀ × *E. ictericus* ♂.

The photographs of these 40 intromittent organs have been placed on the plates in the order of their size, photo 21 being the shortest (60 mm.) and photo 60 the longest (76.5 mm.).

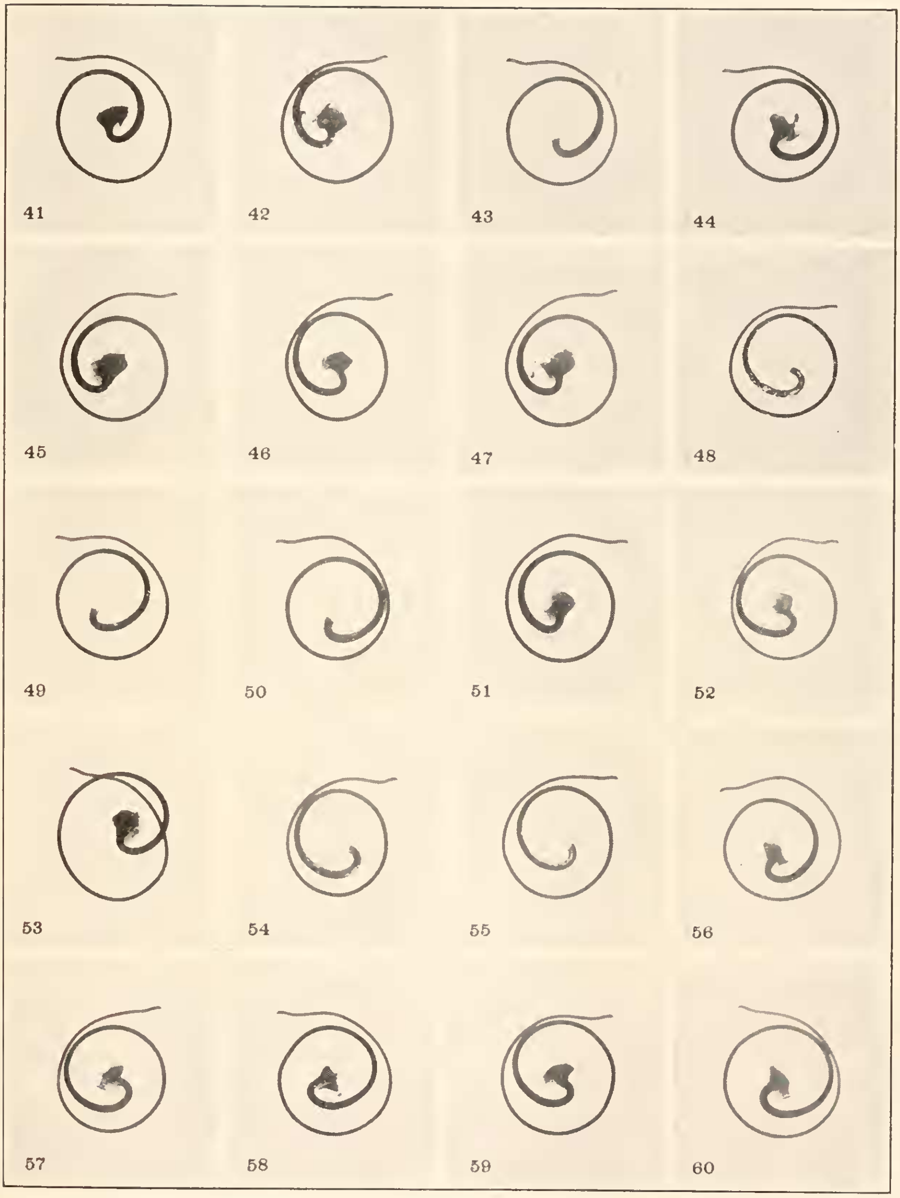
None of the 71 specimens which were measured can be classed with the *variolarius* intromittent organ which varies in length between 85.5 mm. and 106 mm. Ten of the 71, however, can be classed with the *ictericus* intromittent organ which varies in length between 54.5 mm. and 64.5 mm., though none of the specimens are as short as the shortest *ictericus* organ (54.5 mm.). The remaining 61 are variable intermediates.

The length of the intromittent organs of photos 21 to 60 is as follows: Photo 21, 60 mm.; Photo 22, 60 mm.; Photo 23, 62 mm.; Photo 24, 62 mm.; Photo 25, 62.5 mm.; Photo 26, 63.5 mm.; Photo 27, 64 mm.; Photo 28, 64 mm.; Photo 29, 66 mm.; Photo 30, 66 mm.; Photo 31, 66 mm.; Photo 32, 66.5 mm.; Photo 33, 67 mm.; Photo 34, 68 mm.; Photo 35, 68 mm.; Photo 36, 68 mm.; Photo 37, 68 mm.; Photo 38, 69 mm.; Photo 39, 69 mm.; Photo 40, 69 mm.; Photo 41, 69 mm.; Photo 42, 70 mm.; Photo 43, 70 mm.; Photo 44, 70 mm.; Photo 45, 70 mm.; Photo 46, 71 mm.; Photo 47, 71 mm.; Photo 48, 72 mm.; Photo 49, 72 mm.; Photo 50, 72 mm.; Photo 51, 72 mm.; Photo 52, 72 mm.; Photo 53, 73 mm.; Photo 54, 73.5 mm.; Photo 55, 74 mm.; Photo 56, 74 mm.; Photo 57, 75 mm.; Photo 58, 76 mm.; Photo 59, 76 mm.; Photo 60, 76.5 mm.



K.F. & E.C.S. photo.

INTROMITTENT ORGANS of the offspring from the BACK-CROSS, F_1 ♀ (from E. VARIOLARIUS × E. ICTERICUS) by E. ICTERICUS.



K.F. & E.C.S. photo

INTROMITTENT ORGANS of the offspring from the BACK-CROSS, F_1 ♀ (from E. VARIOLARIUS × E. ICTERICUS) by E. ICTERICUS.

PLATE IV.

These photographs of the ventral surface of 41 male insects are of specimens preserved in pure glycerine in test tubes. The genital segment of each insect has been pulled out and cotton inserted behind the segment to hold it in position to show the entire ventral surface.

The magnification is about 1½ diameters.

PHOTO 61. Seven male specimens of *E. variolarius*, showing the typical black spot on the genital segment. These are the same specimens which were photographed for our report of the cross between *E. variolarius* and *E. servus*.

PHOTO 62. Seven male specimens of *E. ictericus*, showing that the spot on the genital segment which is so pronounced in *E. variolarius* (photo 61) is only faintly indicated in *E. ictericus*.

PHOTO 63. The *E. ictericus* male that fertilized the *E. variolarius* female of the first cross, p. 324. Photo 9 shows the intromittent organ of this insect.

PHOTO 64. The *ictericus* male that fertilized the F_1 female hybrid that was raised from the cross *E. variolarius* ♀ × *E. ictericus* ♂.[1] From this back cross (see Record, p. 326) 195 eggs were deposited and 177 of these hatched, 149 were reared to the winged stage (76 males and 73 females).

PHOTOS 65 to 69. Twenty-five specimens of the 76 males from the above mentioned back-cross.

A comparison of these 25 specimens with photo 62 shows the influence of the *E. variolarius* grandmother on the genital spot, for a few of these specimens have the genital spot almost as pronounced as that of the pure *variolarius* species and in the majority of the specimens the spot is stronger than that of the pure *ictericus* individuals of photos 62 and 63 and of the pure *ictericus* parent (photo 64). The *ictericus* inheritance is shown in those individuals which have merely a faint indication of the genital spot, *e. g.*, the lower three insects of photo 67 and the lower two of photo 68.

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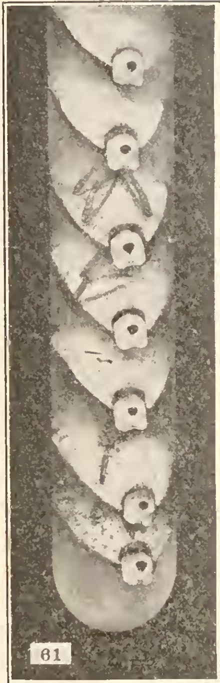
[1] The three apparently pigmented spots on the genital segment of this specimen are misleading,—they are in fact, a slight discoloration of the segment and not a pigmentation. The genital spot of this individual is like that of the lowest specimen of photo 67. Photo 10 shows the intromittent organ of this insect.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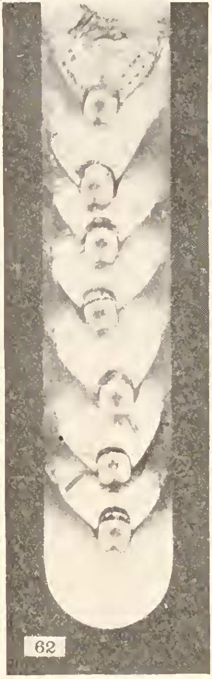
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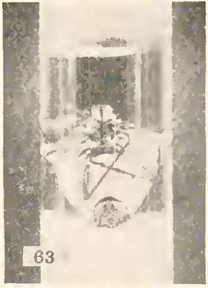
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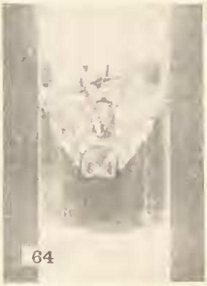
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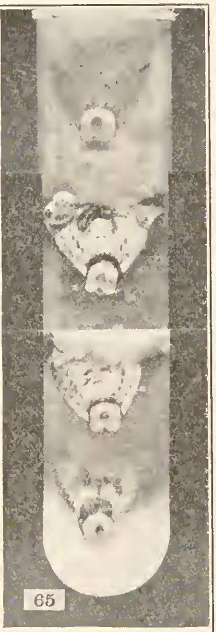
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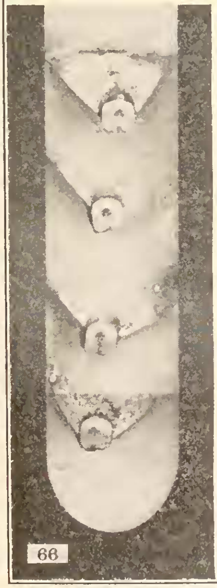
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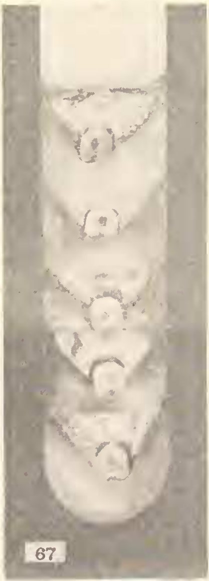
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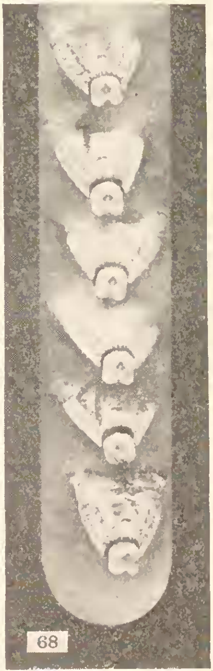
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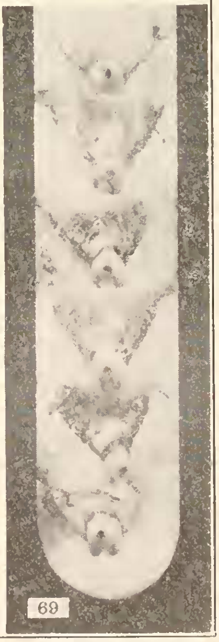
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MALE SPECIMENS of E. VARIOLARIUS, E. ICTERICUS & offspring from the BACK-CROSS, F1 ♀ (from E. VARIOLARIUS × E. ICTERICUS) by E. ICTERICUS.

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A STUDY OF DESICCATION IN THE ROTIFER, PHILODINA ROSEOLA, WITH SPECIAL REFERENCE TO CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

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I. INTRODUCTION.

The investigation upon which this report is based was begun while the author was a graduate student in the Department of Biology of Princeton University. A short summary of the earlier findings has been published in a former article ('14). The study of additional material and the completion of the present paper has been carried out in the zoölogical laboratory in the College of Liberal Arts of Syracuse University during the last year. To Dr. E. G. Conklin, who first suggested this investigation to me, I wish to express my thanks for his interest and for the many suggestions made at various times during the course of this study. I am also indebted to Dr. C. W. Hargitt for many favors during the latter part of my work.

The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. The first recorded observations upon desiccation phenomena are those of von Leeuwenhoek in 1701. From the gutter of a roof he took some dust which he moistened and examined with his microscope. He observed living animals swimming about actively in the water. He found that the animals, which were, no doubt, rotifers, could be deprived of moisture for many months and could then be revived by the addition of water.

After the work of von Leeuwenhoek, the problem of the drying of living things was forgotten for a time. It was not until the period between 1750 and 1775 that interest in the study of desiccation phenomena was revived. During this quarter century many forms were added to the list of animals capable of enduring desiccation.

In 1776 Spallanzani discovered certain tardigrades and nematodes which were able to endure desiccation. He worked also on rotifers and was the first to state that rotifers, when dried free from sand, could not recover from the effects of the drying process. The failure of Spallanzani's rotifers to recover has since been shown to be in no way due to the absence of sand but his experiments aroused much discussion and engaged the attention of many of the foremost naturalists of that day.

Ehrenberg (1838) maintained that the presence of sand protected the rotifers from an actual loss of water. He believed that during the apparent desiccation period all of the vital processes continued, reproduction included. In regard to the function of the sand in retarding desiccation he differed from Spallanzani since the latter believed that the sand protected the rotifer from the injurious effects of the air rather than from loss of water.

Bory St. Vincent, about this time, maintained that under no conditions could the animals survive desiccation but that their apparent revival was due to the hatching of eggs concealed in the sand.

Doyere (1842) after careful study confirmed in the main the observations of Spallanzani. Doyere found, however, that a few rotifers recovered in each lot dried on a clean slide. He disproved the theory of Spallanzani as to the fatal effects of the air, since he found that rotifers dried in air and then placed in a vacuum showed a lower mortality than those dried directly in the vacuum. He concluded that the rapidity of drying is an important factor in the effects of desiccation. He decided from his experiments with rotifers *in vacua* that the last traces of water might be extracted without destroying the power of revival, and that since life processes are impossible in the absence of water, the dried animal possessed life poentially and not actually.

About 1860 a more general interest in the subject of desiccation was revived. The advocates and opponents of the view that desiccation occurs were so evenly divided and the evidence for both views apparently so well founded that the arguments for and against desiccation were considered in 1859 by a commission appointed by the Société de Biologie of France. It was the opinion of this commission that in the dried state life existed only *in potentia*.

Up to this time no one had suggested that rotifers might secrete a water-proof membrane during the dry season. In 1873 Davis said that in *Philodina* there was a secretion of a gelatinous covering which prevented loss of the body fluids. He explained that the presence of sand prevented rapid evaporation of the water and thus gave the rotifers time to secrete the gelatinous envelope before the last traces of moisture on the slide disappeared. This conception has been widely accepted by many up to the present day.

Zacharias (1886) and Faggioli (1891) both reverted to the view of Bory St. Vincent that the supposed revival was due to the hatching of eggs concealed in the sand.

From the time of Faggioli's paper until 1909 no conclusive work on the subject of desiccation appeared. It will at once be seen that the main questions involved in the desiccation problem had not been solved. In 1909 Jacobs, working upon *Philodina roseola* attempted to determine the real conditions attending the drying process.

Jacobs outlines the questions involved in the desiccation problem as follows: "What is the actual effect of drying on a rotifer? Is the water really removed? Does the animal secrete a protecting membrane? If the animal actually dries what is the condition of its tissues? Are life processes at a standstill or are metabolic activities going on all the time in a reduced state?"

Jacobs concluded because of the shrinkage in the tissues, because of negative results in physical and chemical tests for water in the dried animal and as a result of other indirect methods, that the rotifer body becomes truly desiccated. He found no evidence of a waterproof cyst. His answer to the question regarding the condition of the tissues in the dried animal was not very definite, at least not complete, and this for the very good reason that he made no cytological examination of the tissues of the dried rotifers. Jacobs decides that the metabolic changes probably continue in the tissues of dried rotifers.

In all the discussion and observation upon the subject of desiccation phenomena previous to the publication of the preliminary note on this work, apparently no one had considered the problem from a cytological point of view. This is true not only of the study of animal tissues, but among plants as well. Certain algæ, pteridophytes and liverworts can be dried and will subsequently recover. This fact has been known for years. Pfeffer (1903) comments upon physiological phenomena attending desiccation but mentions no changes in structure. The author published in 1914 a preliminary account of the observations recorded in this paper. Brown (1915) working with McDougal at the

Desert Laboratory at Tucson, Ariz., describes cytological changes accompanying desiccation and subsequent recovery in the cells of *Echinocactus wislizeni.* These two papers, as far as is known to the author, comprise the literature on the cytology of desiccation.

Although this paper deals with the same kind of material as was used by Jacobs and although some of the questions asked by him are of most interest here, the problem has been attacked from an entirely different angle and by cytological methods confirms and adds to the results of Jacobs which were obtained by physiological methods.

The purpose of this study is, therefore, to attempt by means of cytological examination to determine (1) the condition of the tissues of the dried rotifer; (2) the presence or absence of a protecting membrane in the dried condition; (3) the condition of metabolic activity in the tissues of the dried animal and (4) the changes attending a recovery from desiccation. In order to do this it will be necessary to compare carefully the cytological appearance of sections of animals in a condition of dryness and those of animals recovering from desiccation with sections of the normal, active animal. From the data so gathered the conclusions will be drawn.

II. Material and Methods.

Philodina roseola is one of the common rotifers belonging to the order Bdelloideæ. It occurs throughout the world and is found in depressions in rocks, eave troughs or almost any place which is moist or periodically moist and dry. It is usually associated with the unicellular alga, *Sphærella lacustris.* Frequently one notices small individuals in hay infusions. These are, no doubt, introduced when dry hay is added to old cultures. Sometimes in infusions rotifers are found which are fully as large as those associated with the alga before mentioned. These may be encouraged to multiply if very weak hay infusion is added from time to time. The weak infusion allows sufficient bacteria to develop to replace the algæ as food and it seems not to harm the animals.

As has been stated by other authors, the behavior of *P. roseola* is dependent upon a number of factors, chief of which seem to be the purity of the medium in which it lives, the amount of food material present and the temperature of the medium. If the water surrounding the animals does not contain too much organic material, they usually may be seen stretched out, fastened by the foot and going through the usual feeding movements. When conditions are unfavorable they contract (Pl. II., Fig. 4) and remain quiet until the surroundings become more favorable. A slight increase in temperature is usually conducive to more vigorous movements, especially swimming.

In preparing *P. roseola* for microscopic examination or subsequent sectioning some difficulty may be experienced because of the fact that the animals are generally associated with sand or other foreign material. When the animals are present in sufficient numbers the method of collection suggested by Jacobs is perhaps the best. This takes advantage of the reaction of the animals to light. If subjected to no mechanical stimulus they usually are indifferent to natural light of ordinary intensity. However, if the culture is violently agitated by shaking or stirring they become temporarily negatively phototropic and may be collected with a pipette from the darker part of the culture. When it was desirable to select individuals of any given size or condition of maturity in these experiments, or to get some absolutely free from foreign material this was accomplished by picking up the animals one by one from a Syracuse watch glass with a capillary pipette, the entire process being watched with a binocular microscope. The foregoing statement is not to be interpreted as meaning that *P. roseola* is ever covered with debris. Such is not the case. The foreign material alluded to consists of bits of sand, etc., to which the animals adhere by means of the sticky secretion of the foot glands.

When first put on the slide under a cover glass the animals are generally so active that a single individual can be kept within the field of a high power lens only with greatest difficulty. As some of the water evaporates from under the cover glass, and as the latter gently presses upon the animal, movements become less rapid and many details of organization can be made out with little trouble.

By putting the rotifer in a weak solution of neutral red some of the internal organs are stained but not with any particular sharpness. By proper manipulation of the light through the condenser the unstained specimen becomes practically as favorable for examination as that stained in neutral red.

In preparing dried animals for sectioning large numbers of active animals, free from sand, were put in Syracuse watch glasses and the latter placed in an oven at about 40 degrees Centigrade. While the desiccation process is always fatal to a few animals in each lot which is being dried, I can affirm what Jacobs has already pointed out, namely, that in the case of animals dried on clean glass the mortality is lowest when the temperature is about 40 degrees Centigrade and the moisture is not allowed to pass off too rapidly.

In preparing undried rotifers for sectioning a large number was collected in a test tube and anesthetized with the cocaine-methyl-alcohol mixture of Rousellet. When the animals were completely relaxed the excess liquid was drawn off and the fixing fluid poured upon the entire mass. After fixation the material was washed thoroughly, passed rapidly through the alcohols, cleared in xylol and embedded in paraffine of 54–56 degrees melting point. Sections were cut 3–5 μ in thickness.

In the case of the dried animals the fixative was poured directly upon them and allowed to act from twenty to thirty minutes.

Several fixing fluids were employed. Bouin's picro-acetic formol was not very satisfactory. A solution of saturated corrosive sublimate with 5 per cent. acetic acid was used hot. This was quite satisfactory in that its action was almost instantaneous and the preservation of structures faithful in most respects. Beauchamp ('09) says that sublimate fixation was very poor in the forms he studied. He does not state the exact formula employed in his experiments but it would appear that he did not try the one mentioned above. By far the best formula for fixation is the osmic-sublimate-bichromate mixture recommended by Beauchamp.

In staining the sections iron-alum hæmatoxylin combined with various counter stains gave the best results. The iron-hæmatoxylin-eosin-lichtgrün stain of Beauchamp is very valuable. Another combination consisting of safranin, orange G and lichtgrün was used with some success. The latter is not so precise as the hæmatoxylin combination.

OBSERVATIONS. 1. *On Entire Animals.*

A. *Anatomy of P. roseola.*—Janson ('93) has carefully considered the anatomy of the Philodinidæ and I find few inaccuracies in his statements as applied to *P. roseola*. It will be necessary, however, to briefly describe the organ systems and their relationships in order to arrive at a thorough understanding of the changes in form and rearrangement of parts incident to the desiccation process.

The body of *P. roseola*, at first sight, suggests that of a segmented worm. Its segmentation (which affects only the integument and accessory structures) is, however, not so regular but that the body is easily divided into head, trunk and foot. Its outer integument is formed of a thin, more or less transparent cuticle beneath which is a granular plasma layer, the hypodermis. In the hypodermis are deposited pigment granules. These are absent in the very young animal and occur in varying number in the adult. The cuticle is soft and flexible, easily lending itself to the telescoping and folding so characteristic of this *Philodina*.

The hypodermis is a syncytium which contains nuclei distributed evenly through it. There are, in the mid-body region, longitudinal folds of the skin which are always present. Temporary cross foldings occur as a result of the vigorous movements of the animal. Externally the skin lacks spines or protuberances of any kind.

The head, whose most prominent structures are the trochal discs, extends as far back as the forward margin of the mastax. When the trochal discs are folded in, a blunt, ciliated projection is seen at the anterior end. This is the proboscis and it is used for tactile purposes as well as to aid in the "measuring-worm" type of locomotion so characteristic of the Bdelloideæ. The rotating organs consist of a double row of cilia, broken in the case of the anterior row, in the ventral part of the head. The more posterior ciliary wreath is made up of shorter elements and is directly continuous with the cilia which line the pharangeal cavity. The hypodermis in the head region at the base of the trochal discs is thickened, probably to furnish additional support for the rotating apparatus, and in some specimens the nuclei of this layer can be seen near the bases of the lines of cilia.

Dorsally, and arising at the posterior margin of the head, is the tactile organ (Pl. I., Fig. 1, *t. o.*) which shows from two to four segments according to its state of extension. It is capped at the end by a tuft of short cilia. Nerve fibers connect it with the brain as Zelinka has shown.

The brain (Fig. 1, *br.*) lies with its anterior margin just at the base of the tactile organ, while it extends posteriorly slightly over the forward part of the mastax. It is triangular in shape with the sharpest angle extending forward. The eyes (Fig. 1, *e*) which are red in color, lie dorsal to the brain.

The excretory canals (Fig. 2, *e. c.*) extend into the head and a terminal flame cell (*f. c.*) can sometimes be seen beating at the base of each trochal disc. These flame cells are perhaps best seen when the trochal discs are folded in. They appear very close to the margins of the folded discs (Pl. II., Fig. 3).

The trunk is almost entirely filled with the organs of digestion and those of reproduction. In its most anterior part the mastax (Pl. I., Fig. 1, *m*) is prominent. Food enters this by way of the narrow, ciliated pharanyx (Pl. I., Fig. 2, *ph.*). The mastax proper consists of two more or less crescent-shaped jaws. Transverse ridges, which at the inner margins continue as short projections, form the teeth (Pl. I., Fig. 1, *t*). In all the animals I have studied there have been two teeth on each jaw. A few authors report cases where there are three teeth on one jaw and two on the other.

The mastax is surrounded by glands which probably contribute a digestive fluid to the food as it is being crushed in the mastax. There are five of these salivary glands, two of which lie dorsally and three ventrally. The dorsal ones (Fig. 1, *s. g.*) follow the outline of the mastax quite closely, while those on the ventral side are usually larger and extend posteriorly (Fig. 2, *s. g.*). In the living animal these glands show a granular cytoplasm with nuclei of varying sizes. These nuclei appear as bright spots against the darker cytoplasmic background.

From the mastax a narrow esophagus leads to the stomach-intestine (Pl. I., Fig. 1, *st. i.*). In most cases it is difficult to see the esophagus in the living animal. This is also true of a pair of glands which lie at the anterior end of the stomach-intestine. Both the esophagus and these glands are obscured by the ventrally lying salivary glands, and it is only when the animal is thoroughly stretched that these structures can be distinguished.

The stomach-intestine, as seen in the living animal, consists of a yellow, viscous tissue containing granules, fat-droplets and nuclei. By careful observation all of these elements may be recognized. The lumen is usually not seen except in sections. In healthy, active animals the stomach tissue shows its color distinctly. In starved animals the color is lacking in the stomach tissue as Zelinka has pointed out. In newly hatched *Philodina* the stomach is almost perfectly transparent.

Just posterior to the stomach-intestine is the "blasendarm" (Fig. 1, *bl.*) of Zelinka. This is thick-walled but not glandular. It serves for the accumulation of the undigested remains of the food. By the contractions of its walls this material is evacuated from time to time. Darker in color and more homogeneous in structure, this first division of the end gut is the most prominent structure in the posterior part of the trunk of the living *Philodina*.

The "blasendarm" leads into the rectum (Fig. 1, *r*), a narrower tube in the dorsal wall of which the contractile bladder (Fig. 1, *c. b.*) is found. This bladder is thin-walled, extensible and on either side receives the lateral excretory canals. The bladder pulsates at intervals of fifteen to twenty seconds and in so doing disposes of the accumulated waste which has been emptied into it by the excretory canals.

Lying on either side of the stomach-intestine are the reproductive organs. Since no males of *Philodina* are known, these organs are always ovaries with their accessory structures. The ovary consists of from six to ten small nuclei which lie close together in a clear syncytial ground substance on the inner margin of the vitellarium. It is not easily seen in living animals unless it contains a developing egg (Pl. I., Fig. 1, *eg.*).

The vitellarium (Fig. 1, *v*) is especially prominent in living animals. It is a spindle-shaped structure at either end of which is a connective tissue strand which fastens it to the other organs. The anterior strand is fastened to the body wall in the region of the mastax, the posterior one attaches to the digestive system in the region of the boundary between the "blasendarm" and contractile bladder. The posterior strand looks as if it might act as an oviduct but I have never seen an egg or an embryo passing through it. This fact, together with observations of other authors who have seen embryos of *Philodina* liberated from the mother by a rupture of the body wall, makes it seem certain that if this structure ever did function as an oviduct it is now only rudimentary. The vitellarium contains eight large nuclei arranged in a row. This number may vary but this is not usually the case.

The lateral excretory canals run posteriorly through the trunk and very close to the vitellaria. They are minute, thread-like structures having flame cells connected to them at intervals. In *P. roseola* I have never observed more than five of these flame cells on each side. The canals empty into the contractile bladder as was described above.

The rectum is a narrow tube leading posteriorly from the contractile bladder and terminating dorsally in the anus (Fig. 1, *an.*) at the posterior border of the second foot segment.

The foot contains the glands which secrete a sticky substance which enables the animal to fix itself during its crawling movements. These glands are oval in outline, are uninuclear and empty by means of ducts at the base of the toes. When the animal is folded up these glands become packed closely about the "blasendarm." When the rotifer stretches out they occupy places in the four posterior foot segments. There are four toes on the tip of the foot. These aid in fixing the animal during feeding movements.

B. *The Active, Free-swimming Animal.—P. roseola* in its behavior shows no great difference from most of the other Philodinidæ. Zelinka ('86) and Janson ('93) have described the movements of the philodinids in general terms while Jacobs ('09) has given an account of *P. roseola* both under ordinary conditions and at the onset of desiccation. I have given the behavior of *P. roseola* much attention during the course of this work and am able to confirm Jacobs in all essential respects.

Two methods of locomotion are employed, viz., swimming by means of the trochal cilia and creeping by the alternate use of foot and proboscis. The method of locomotion depends to a large extent upon the temperature and purity of water in which the animal lives. Other things being equal, a relatively high temperature is conducive to active swimming movements, while with lower temperatures the animal generally restricts itself to the leech-like creeping. Another factor influencing the kind of movement is the purity of the water. In cultures which contain many putrefactive bacteria the animals usually are found creeping sluggishly about, and they seldom extend the trochal cilia for any great length of time.

Swimming is the result of the rotating of the trochal cilia and involves no turning upon the body axis. The body movements during swimming are similar to those of certain infusoria as described by Jennings ('04). If any obstruction is met with in swimming the trochal cilia are suddenly retracted and a series of random testing movements occur. The cilia are then extended and swimming is resumed in a direction where there is no obstruction. Sometimes an animal will be observed to stop if it swims through a region where food is abundant. In this case it attaches itself by the foot and feeding movements begin.

In creeping the rotating organs are always retracted. The animal attaches itself by means of the secretion of the pedal glands and then after a greater or less number of testing movements, in which the body is stretched successively in several directions, it attaches itself by the end of the proboscis. This attachment of the head region is followed by the wrenching loose of the foot. The latter is then brought to a point nearer the head and the operation is complete. In changing from the creeping to the swimming movement the trochal discs are extended and the foot is loosened by a contraction of the muscles in the posterior region. The animal then moves steadily forward as long as its trochal cilia are in motion.

While feeding the animal is always attached by the foot. Currents created by the rotating organs carry bacteria, algæ, etc., in a steady stream into the open pharynx where they are further propelled by the long cilia lining the pharynx. The body may sway from side to side during feeding, in fact, the animal is frequently seen to bend its body as if to reach some particles which were not before influenced by its trochal activity. During all this time the mastax is active. Its grinding operations are easily observed in any feeding animal.

C. *The Contracted Animal.*—Zelinka ('86) in describing the movements of rotifers of the genus *Callidina* states that the animals frequently retract the head and rotating organs without any apparent reason. He observes further that their subsequent extension may be rapid or slow and that no definite reason can be assigned for the lack of uniformity in this respect among different individuals. *P. roseola* contracts very rapidly but usually extends itself slowly. The factors which influence it to contract seem to be (1) Sudden change in temperature of the medium; (2) change in the chemical composition of the medium; (3) mechanical stimuli; and (4) desiccation.

Gradual changes in temperature in the medium in which the *Philodina* lives do not cause it to contract. Increase in temperature is conducive to more vigorous movements while a decrease causes the animal to become sluggish. If the temperature is suddenly changed by the addition of hot or cold water the animal contracts and does not extend itself for many minutes.

The addition of any active chemical to the culture causes the rotifers to suddenly contract and if the foreign substance is not removed they will remain in the contracted condition until death ensues. Philodinas kept in infusions will frequently be found to become inactive in this way. An examination usually shows that the infusion is in too great concentration or that putrefactive or acid forming bacteria have rendered the environment unfavorable.

A mechanical shock of any kind will make the animals contract completely but a contraction under such conditions usually lasts but a few seconds unless the stimulus is continuous or repeated.

When the water around a *Philodina* begins to dry up the animal creeps about rapidly apparently trying to escape from the diminishing drop. This creeping continues until movement is no longer possible. The animal then contracts into an almost spherical mass and dries.

Especially interesting to observe is the manner in which the organs are arranged in the contracted animal. While in the extended animal there are spaces between organs, when contraction takes place every available bit of space inside the trunk integument is filled by the closely packed organs. The head and foot segments are drawn entirely within the trunk by means of the contraction of longitudinal muscles. The circular muscles at each end of the trunk may then contract and cause the entire animal to assume a shape not unlike a lemon. The large organ systems can be seen through the integument of the contracted rotifer. The head and its accessory structures can be seen just anterior to the stomach tissue. The stomach occupies the central part of the mass and is flanked on either side by the reproductive organs. In the posterior end of the trunk the foot segments containing the pedal glands can be seen. Fig. 4, Plate II., shows a *Philodina* which has been contracted but is just beginning to unfold. The foot segments are slightly protruded while the head still remains within the trunk cavity.

D. *The Dried Animal.*—When the drop of water surrounding a *Philodina* begins to dry up the animal indulges in active creeping movements until the drop becomes too small to permit further activity. The rotifer then contracts and actual desiccation begins. It is at this time, according to the older authors, that the jelly membrane is secreted. The shiny appearance described by them is indeed apparent at times but this is due not to any jelly but to the fact that the tissues of the rotifers become packed together as water disappears and since loss of transparency accompanies loss of water the light is reflected rather than transmitted by the animal. Final proof of the absence of a jelly layer in dried rotifers will be given in the part dealing with sections.

The internal organs cannot be carefully studied while the drying is going on since the integument usually folds and wrinkles as soon as drying begins. This obscures the internal structures.

Animals dried under favorable conditions all tend to assume a similar shape. The head and foot segments are drawn into the trunk as before described. The circular muscles at the end of the trunk contract giving to the animal a spindle shape. When the drying process is very slow the irregular wrinkles do not appear but the puckering due to the contraction of the circular muscles is evident. Jacobs has pointed out this fact and my Fig. 16, a longitudinal section through a dried animal, shows the sides as being free from folds while the ends show prominent and regular wrinkles.

Frequently several animals are carried together by the diminishing drop as it evaporates. In this case each rotifer tends to assume a hexagonal shape as a result of mutual pressure. Fig. 5, Plate II., shows an animal which was dried on a clean slide at room temperature and drawn nine days after the desiccation began. It will be seen at once that the integument has become much folded and the internal organs indistinct, but the vitellarium, foot and stomach are still recognizable.

E. *Animals Recovering from Desiccation.*—When water is added to dried animals, such as that described in the last section, they usually regain their normal size within ten minutes, often before. The rapid swelling of the animal causes any wrinkles which may be in the integument to disappear. Active movements may occur within a short time if the conditions under which they are dried are favorable. Sometimes movements may not occur for several hours, even a whole day. This fact may account for the negative results of the desiccation experiments of some of the older authors. These investigators probably did not give their rotifers sufficient time to recover, as Jacobs points out.

As the animals recover they resume their usual activities. I have noticed that rotifers recovering from desiccation are lighter in color than they were before the process began. Further comment will be made upon this point in a later paragraph.

2. *Study of Sections.*

The cytological details of the structure of rotifers has been described in comparatively few cases. Of the Bdelloideæ, *Discopus synaptæ* has been described by Zelinka ('86). From the study of the figures of sections through the principal body regions as represented by Zelinka one can see many points of similarity between this form and *P. roseola*. In the case of *P. roseola* I find no record in the literature which tells of the cytological structure of its tissues. The accompanying figures and descriptions are therefore presented here for the first time.

The finer cytological changes accompanying the process of desiccation and subsequent recovery can only be shown after describing the conditions present in a normal, undried specimen. I shall therefore now describe the arrangement of organs and their cytological peculiarities in both extended and contracted normal animals, after which comparisons will be made with sections of animals which have been desiccated and also with sections of animals recovering from desiccation.

A. *The Normal Extended Animal.*—The relationship of the internal organs is well shown in transverse sections of the extended *Philodina*. Fig. 7, Plate III., shows a section through the posterior part of the head. The pharyngeal cavity leading to the mastax is lined with cilia. Its wall consists of thick cells with large nuclei. The brain, with outer cellular elements and inner homogeneous zone, appears close to the pharyngeal tube. The integument surrounding the region of this section is much thinner than it is in the mid-body region. This is to be expected when one remembers the flexibility of the head segments as compared with those of the trunk. There is a marked similarity in the arrangement of the elements here described and those in a transverse section through the anterior part of *Discopus synaptæ* as described by Zelinka ('88).

In *Discopus*, Zelinka shows the integument as being much thinner than I have found it in *Philodina*. The hypodermis of *Philodina* appears as a definite layer much thicker than the cuticle and containing nuclei embedded in its syncytial groundwork. This feature is seen in the figures of the sections throughout all parts of the animal's body. In *Discopus* the nuclei are flattened rather than round and appear as swellings upon the cuticle. Furthermore, Zelinka figures the hypodermal nuclei as being without a nucleolus. Such a condition is not usually found in *Philodina*. Another point of variance between the two forms concerns the brain. Zelinka figures the brain of *Discopus* as being composed of an outer ganglionic layer and a central "punktsubstanz." In *Philodina* the same divisions appear but the cells of the ganglionic layer have distinct walls as distinguished from the syncytial condition in the brain of *Discopus*. The walls of the pharynx with their internal ciliation are not markedly different in the two forms.

A section through the esophagus is shown in Fig. 8, Plate III. As was mentioned before, the esophagus is difficult to observe in the living animal because it is generally concealed by the salivary glands which surround it. The section shows it to be a narrow, thin-walled tube. Surrounding it are the salivary glands whose cytoplasm is uniformly granular but not divided into distinct cells. The nuclei appear scattered irregularly and have the characteristic structure found in like elements of other tissues.

The esophagus in section in *Discopus* shows about the same relative size and position as it does in *Philodina*. It is likewise surrounded by the salivary glands so that it probably is not visible in the living animal. The salivary glands of *Discopus* do not appear to be as dense or deeply staining as those of *Philodina*. The cytoplasmic granules are less closely packed together and the nuclei appear to be less chromatic. The most noticeable difference between the figures of sections of corresponding regions in the two animals is the lack of hypodermal tissue in *Discopus*. While it is to be expected that the integumentary structures would be thinnest at the points of greatest flexibility, it seems that the total absence of hypodermis in this and some other of Zelinka's figures must have been a result of an oversight on his part.

A section through the middle portion of the trunk (Fig. 9, Plate III.) shows the reproductive glands and the middle portion of the digestive system surrounded by a somewhat thicker ring of the integument. The plasma of the vitellarium is a syncytium made up of granules of varying sizes. The granules are surrounded by small, clear areas containing a cell-sap which is lost during desiccation. The nuclei of the vitellaria (*v*) are the largest to be found in the rotifer body. These consist of a single karyosome surrounded by a clear homogeneous area and having at the periphery a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and is characteristic of most of the cells of the rotifer tissues. The ovary (*ov*) is a small elongated structure lying in a depression of the inner border of the vitellarium. It never attains very great size. It consists of from five to twelve intensely chromatic nuclei surrounded by a glassy, homogeneous plasma. A section of a typical ovary is shown in Fig. 10, Plate III. Both ovary and vitellarium are surrounded by a delicate membrane.

Zelinka does not figure a section of the ovary of *Discopus* containing more than four nuclei. This small number is frequently observed in *Philodina* but the average number is slightly larger. The clear, non-staining cytoplasmic portion of the ovary seems to be similar in both forms but Zelinka does not figure a membrane separating ovary and vitellarium. Whether this last point was due to improper or insufficient staining is hard to say but it seems that a limiting membrane should be present. In *Philodina* a definite ovarian capsule can be easily demonstrated in sections through the proper region of the normal, free-swimming animals.

Another point which leads me to believe that the staining methods employed by Zelinka were insufficient to demonstrate all structural details is the fact that he does not figure a nuclear membrane in the nuclei of the vitellarium. The karyosome is surrounded by a clear area as in *Philodina* but he figures no limiting membrane about the entire structure. It is possible that he thought the karyosome to represent the entire nucleus. However this may be, there is, in all cases, in the vitellarium of *Philodina* a definite nuclear membrane surrounding the clear, outer zone of the nucleus.

In cross-section the stomach-intestine (Plate III., Fig. 9, *st*) shows as a thick-walled tube with a narrow lumen. From the inner wall cilia project into the lumen while at the base of the cilia are deeply staining granules, which Beauchamp, in other rotifers, interprets as cross-sections of longitudinal muscle fibers. My own observations lead me to believe that this interpretation is correct. The stomach tissue is of a spongy consistency, the ground substance appearing as closely packed vacuoles. No cell walls can be demonstrated but nuclei are scattered at intervals throughout its extent. The nucleus has a karyosome surrounded by a homogeneous plasma which stains with acid dyes. Scattered irregularly through the stomach tissue are large, deeply staining granules which are masses of reserve food material. Janson ('93) says that these masses are kept by the rotifer for use during the dry periods when feeding must of necessity cease. Beauchamp ('09) shows sections of the stomach of *Hydatina senta* in which these granules appear. He shows further that in a section of the stomach of an individual of the same species which had been starved for twenty-two days, these granules had disappeared and were replaced by vacuoles. In sections of very young *Philodinas* no such granules are present and I shall show later that after periods of desiccation in *Philodina* these granules become fewer or disappear entirely, thus furnishing cytological evidence in support of the theory of Janson and Zelinka, which by them was based entirely upon observations upon the entire, living rotifers.

The stomach tissue is most pliable and the lumen seems normally to be able to occupy almost any position in it. This fact has been remarked by Beauchamp in *Callidina socialis.*

The stomach tissue of *Discopus* as figured by Zelinka lacks some of the elements which I find in *Philodina.* I refer to the granules of reserve food material just mentioned. In none of his figures does he show these deeply staining aggregations. It may be that the digestive processes of *Discopus* differ from those of *Philodina.* The difference in the habits of the two forms might account for this. On the other hand these elements might have been present but not differentiated by the stain. The latter condition does not seem probable, however, for I have found that most of the nuclear stains have an affinity for the food granules.

Another point of difference is in the structure of the ground substance of the stomach tissue. In *Discopus* this ground substance is represented by Zelinka as being made up of fine granules loosely packed together. In *Philodina* the ground substance of the stomach tissue is composed of alveoles closely apposed. This difference could perhaps be accounted for upon the basis of fixation.

Fig. 11, Plate III., shows a section through the most posterior part of the trunk at the point where the stomach-intestine joins the "blasendarm." The contrast in the structure of the two tissues is well marked. The "blasendarm" contains none of the reserve food granules and its plasma has only occasional nuclei scattered through it. Zelinka does not show a section through this region of *Discopus* but there is probably no great difference here between the two animals.

Fig. 12, Plate III., is a transverse section through the second foot segment. It shows the pedal gland (*f. g.*) whose cytoplasm consists of fine alveoles and whose cell-walls are distinct. A single nucleus is present in each cell. The form of this nucleus is subject to little variation in the glands of the undried animal. It consists of a karyosome surrounded by a clear space and a well defined nuclear membrane.

The foot-glands of *Discopus* differ but slightly from those just described. The individual cells are perhaps more nearly round than those of *Philodina* and the chromatic part of the nucleus more nearly spherical. The general arrangement and appearance of these cells is, however, not much different from those of *Philodina*.

Ventral to the pedal glands and separated from them by a narrow space, lies the cloacal cavity. This section throws some light upon the nature of the contractile bladder itself. It has been assumed by Huxley, Claus, Vogt and Yung, Hudson and Gosse, that the contractile bladder is merely the enlarged ending of the lateral canals and must therefore be considered as a part of the excretory system in a strict sense. Semper thought that the contractile bladder merely forced the excretory fluid into the end-gut and that other contractions of the cloacal wall were necessary before the fluid reached the exterior.

In Fig. 12 the cloacal chamber is shown to be composed dorsally of a thin membrane such as one would expect in a structure as distensible as the contractile bladder is known to be. Ventrally the wall is thicker and in all respects similar in its texture to that of the "blasendarm" (Fig. 11). On one side and at the junction of the thin dorsal and thick ventral walls there is a break which I interpret as the entrance of the excretory canal. The large cell lying beside the entrance of this canal is part of the sphincter which prevents the fluid from re-entering the lateral canals at the time of the contractions of the bladder.

This sphincter has been described by other authors but sections of it in *Philodina* have never been shown.

It is evident then, that the contractile bladder is not an independent structure connected to the posterior part of the gut by a narrow neck but is only a portion of the wall of the gut modified to permit its distension. Its periodic contractions force the collected liquid waste to the exterior without further contractions of other parts of the cloaca. The more solid waste from the digestive canal is forced out of the "blasendarm" by independent contractions of the latter structure.

B. *The Contracted Animal.*—The closely packed condition of the organs in the folded animal is shown in Fig. 13, Plate IV. The cilia of the trochal discs and some of those lining the pharynx will be noticed. On either side of the infolded trochal cilia are the ends of the large glands connected with the mastax and with the anterior end of the stomach-intestine. The edge of the chewing apparatus is shown in the middle of the section, while surrounding it will be seen the stomach tissue and a small part of its lumen. The folding of the integument of the animal is well shown here, especially at the anterior end where the convergence of the longitudinal folds shows in section as a rosette. It is apparent that in the contracted condition most of the water which circulates in the body spaces is lost, for the organs lie close to each other in contrast to the condition shown in Figs. 1, 2 and 3 where the spaces, especially those between the integument and internal organs, are very large. Fig. 14, Plate IV., shows a section through a slightly different plane in an undried, contracted animal. In the central part of the section the cells of the outer part of the brain are shown while around the periphery various gland cells appear. This section also goes through one of the vitellaria and shows its characteristic form and structure.

C. *The Dried Animal.*—In the desiccated animal the arrangement of organs is in no way different from that in the previously described contracted, normal ones. The organs are still more closely apposed and the folding of the integument is closer and better marked.

Fig. 16, Plate IV., shows a section of a rotifer which was dried at room temperature and then kept in an evacuated calcium chloride desiccator for eighteen days. At the end of this time it was fixed and sectioned. It will be observed that the cilia still show as individual fibers and have not fused into a homogeneous mass as some authors assert must be the case when all moisture is removed. There is no sign of fusion or other abnormal condition in these elements. Each cilium preserves its identity as well as would those of an animal living in its natural environment.

Of especial interest is the structure of the integument in the dried state. It has been asserted, and for some time quite generally believed, that just before actual drying takes place the rotifer secretes a jelly-like capsule through pores in the skin and this capsule hardens to make a water-proof cyst which remains during the dry season and is dissolved again upon the addition of water. Janson ('93) thinks it is not unreasonable to suppose that a gelatinous covering is secreted. He reasons that since the pedal glands (which are undoubtedly derived from the hypodermis) are known to secrete a sticky substance and since the tube-dwelling forms secrete a slime from their skins which helps to make the tube, then the forms which survive desiccation might easily do the same. He admits, however, that he has not found the actual secreting tissues.

Jacobs ('09) found, as a result of staining reactions with methylene blue, that the integument in the trunk is undoubtedly different in its chemical nature from that of the head and foot. He says: "The fact that that part of the cuticle which alone is exposed at the time of drying should be of a different nature from the remainder is probably significant." Jacobs is convinced that no water-proof cyst is secreted and he suggests that the thickened integument of the trunk region may be a means of preventing a too rapid evaporation as dryness comes on.

While I have no cytological evidence to show that the suggestion of Jacobs is correct or incorrect, certain it is that there is no thickening by a secretion or otherwise of the integument in the dried condition. Fig. 16 shows that instead of the integument being thicker it is actually much thinner than it was in the undried condition. The cuticle shows no great change in thickness. This would be expected in a non-protoplasmic structure. The hypodermis, however, which in the undried animal is from two to four times as thick as the cuticle, has shrunk until it is scarcely thicker than the cuticle itself. The nuclei do not shrink perceptibly but cause swellings on the hypodermis at the points where they occur. I have examined sections of hundreds of specimens dried in various ways and know that this is the characteristic condition of the integument of the dried rotifer. I believe that these observations should effectively dispose of the arguments of those who maintain that the drying animal secretes a water-proof cyst for protection during the dry periods.

In the vitellaria the drying process affects the cytoplasmic portion in less striking fashion than it does the nuclei. The granular material of the cytoplasm appears almost the same as in the undried organs. The spaces between granules are less noticeable. These spaces are probably filled with cell-sap in the active animal and it is to be expected that with loss of water they will largely disappear, permitting the more solid granules to pack closely together. The membrane surrounding the vitellarium and ovary shows no marked change. It is of practically the same thickness and consistency in the dried as in the undried animal.

In the nuclei of the vitellarium noticeable changes have occurred. As was stated before, a section of the nucleus in the vitellarium of an undried animal shows a central, densely staining karyosome surrounded by a clear space, and around the clear space a definite, but not usually thick, nuclear membrane. The nucleus in the dried vitellarium loses, for the most part, its affinity for stains. The karyosome may entirely disappear but usually there are remnants of it distributed here and there through the nuclear space. Taking the place of the karyosome is a more or less regular reticulum which reaches to the nuclear wall. The latter has become thickened during the process of drying; whereas in the nucleus in the undried condition the densely staining material was aggregated in the center and the clear area around the periphery, in the nucleus in the dried condition these relationships are just reversed. What remains of the chromatic material has collected close to the nuclear wall and the central area shows a more or less clear condition. This change in the position of the chromatic material in the nucleus is not the only one incident to the drying process in the vitellarium or in the other organs as a comparison of the figures will show, but it is by all means more frequent than the other changes.

The condition of the ovary in the dried animal is best shown in Fig. 21, Plate V. It will be remembered that the cytoplasm of the undried ovary usually shows as a glassy, homogeneous ground substance and that the nuclei appear as dense, spherical bodies closely packed in this syncytial ovarian cytoplasm. In the dried ovary the cytoplasm becomes more deeply staining and assumes an appearance not unlike that of the vitellarium. The nuclei become less dense and the chromatic material collects in a peripheral ring as was described for the vitellarium nucleus.

In Fig. 21, Plate V., a trough-like depression is seen at the margin of the vitellarium which lies nearest the integument. This is caused by one of the band-like, circular muscles, which, drying in the contracted condition, caused a deformation in the vitellarium tissue which shows thus in longitudinal section.

At this point it might be well to comment upon the fact that many of the muscles in the rotifer body actually dry while contracted. It would seem that when the normal moisture content was removed from a muscle that it would have a tendency to relax and in this way cause the animal to become more loosely folded during the later stages of drying. That this is not true is evident from the figures and descriptions given. The rotifer remains tightly contracted during the most complete conditions of desiccation.

This condition may be due to two factors. In the first place the integument is the first structure to dry. The moisture evaporates from the surface more quickly than from the internal structures. This integument is chitinous in its outer layer but is of such consistency that while moist it is very pliable. When the moisture is removed, however, the cuticle becomes more rigid. In this respect it might be likened to gelatin and such a resemblance is indeed noticeable. Now with the withdrawal of moisture the cuticle could form a rigid capsule which would retain the original shape of the folded animal even if the muscles did relax.

On the other hand it would seem that if the maintenance of the shape of the folded animal was due to the action of the cuticle the muscles would be somewhat flattened in cross-section as a result of the mutual pressure of internal organs and integument. This last condition, however, is not true, for the muscles in the dried animal still show in cross-section something of their oval outline. It seems probable that there is a limit to the amount of drying which a muscle may undergo and at the same time continue to exert a contractile tension. But the amount of drying necessary to cause a muscle to reach this limit would at the same time withdraw enough water from the cuticle to cause it to become rigid. I think, therefore, that we can account for the lack of relaxation at the time of drying by assuming that both of the factors mentioned above acting in succession produce the given result.

The appearance of the mastax and its surrounding salivary glands in the dried animal is shown in the central part of Fig. 16, but perhaps better in Fig. 25. Upon comparison with Fig. 24, which is a section of the undried mastax and glands, it will be seen that the entire structure has collapsed and decreased perceptibly in size. The gland tissue is more dense as to its cytoplasmic content while the nuclei, although just as prominent as before, show the same rearrangement of chromatic material as was described for the nuclei of other tissues in an earlier paragraph. The details of this chromatic movement given for the nuclei of the vitellarium and ovary will apply here and need not be repeated. The same is true of the foot-gland cells although I have observed certain cases where the nuclei of the latter do not assume the usual ring form. This condition is shown in Fig. 19.

The stomach of the dried animal shows, perhaps, the most remarkable changes. In Fig. 16, lying below and at the side of the mastax, is figured the stomach in its dried condition. The cytoplasmic syncytium still preserves its alveolar appearance. The lumen is shown winding about through the syncytium, while nuclei, made up of the chromatic ring, with central clear space, are scattered irregularly about. The densely staining granules of food material, so prominent in the sections of the undried stomach, are not present here. I have examined large numbers of sections of dried animals, and while the food granules are not always entirely absent, as figured here, yet they are absent in many cases, and certainly in all cases they are much less numerous than in sections of the stomach of undried animals. This point is of importance as bearing upon the question of metabolism in the dried state. As was previously stated, the identity of these granules as particles of food material can hardly be questioned. Zelinka ('88) has called attention to the fact that they do not appear in sections of the stomach of the newly hatched rotifers. Beauchamp ('09) has shown that in sections of the stomach of *Hydatina senta* which had been feeding regularly these granules were present in great numbers, but in sections of the stomach of starved individuals they do not appear. Many authors have commented upon the fact that among those rotifers which survive desiccation the stomach tissue in gross appearance is generally lighter in color after recovery than it was before drying commenced. This observation I have repeated many times and find it to be correct. I have not, however, been able to find in the literature figures of actual sections of the stomach of rotifers recovering from desiccation. Fig. 16, which is but one of many that I might show, by the absence of all food particles demonstrates that metabolic or at least katabolic activities must go on in the stomach tissue of the dried animal.

Of great importance in connection with the question of the nuclear changes is the determination of the exact time at which the chromatic rearrangement takes place. It is a reasonable supposition that it could not easily be brought about while the moisture is entirely absent from the tissues. Since the protoplasm is always more or less fluid in nature in its normal condition, the changes described would certainly have to take place very slowly if they occurred in the dried state. It would seem, upon theoretical grounds, that the more favorable time for the changes to take place would be during the short period just before actual drying occurs. At this time the tissues contain the usual amount of moisture but the approach of the dry condition is, at the same time, apparent to the animals. That there can be no doubt about the preparation of the animals for the dry state is evidenced by their behavior at the onset of desiccation, before described.

Sections of animals killed at the moment when the last visible traces of moisture were disappearing, confirm the supposition outlined above. The nuclei of the cells of the different tissues plainly show the remarkable changes which are going on and which I have described in an early part of this section. While in many cases the rearrangement of chromatin is not so complete as in some of the sections of dried animals here shown, yet it was definite and uniform in the different tissues and indicates beyond question that the transitional period for chromatic rearrangement occurs just before the last traces of moisture are removed.

The movement of chromatin just before drying is interesting for the light it throws upon the question of the lability of the nuclear material. Chromatin undergoes changes of form, position and chemical structure at the time of mitotic activity but there are few cases recorded where such changes occur during the resting condition. In the present instance, however, we have a marvelous rearrangement of the nuclear materials which occurs as a vegetative rather than a reproductive process, the essential steps of which may take place in a few minutes and the new internal conditions so established enabling the animal to resist an unfavorable environment for years.

In this connection also lies a clue to the solution of the question of mortality among rotifers which have been dried. Since under the most favorable conditions of drying some few animals never survive, it seems that there should be some definite cause to account for the fact. Comment has been made concerning the tearing of the organs of a rotifer which might result from too rapid drying. In cases where this last factor cannot enter there are still some fatalities and these cases seem to admit of explanation upon the basis of lack of time or vitality to bring about the internal rearrangement of cell elements necessary in resisting the dried condition.

A further confirmation of the fact that the changes in cell-structure take place early in the desiccation process is found by comparing sections of animals which were dried and kept in the open air with those of animals kept days or weeks in an evacuated desiccator. The essential features of cell structure and arrangement are the same in both cases. This certainly seems to indicate that the extent of the adaptive, structural response to the new condition is not directly proportional to the intensity of the stimulus.

The nuclear membrane undergoes no disintegration or other visible change in structure during the drying process. It might be supposed, from a consideration of the movements of the chromatic material into the cytoplasm, that the membrane would break up during the process. Such is not the case. Not only does it remain intact during the drying but it actually thickens. This thickening is of course due to the migration of chromatic material which normally occupies the middle of the nucleus. The chromatin proper cannot be distinguished from the nuclear membrane, however, since both react alike to stains. The nuclear membrane, then, is a persistent cell organ and does not atrophy as a result of the abnormal conditions.

It is interesting to speculate whether the material which the nucleus imbibes upon recovery from desiccation is the same as that which passed through the nuclear membrane at the time of drying. I have not been able to make any of the finer microchemical tests upon this substance but it would seem that the material which passes from nucleus to cytoplasm takes part in the oxidations discussed in a later section. The chromatin remaining in the nucleus probably manufactures new nuclear material from elements imbibed from the cytoplasm upon addition of water.

The conditions described in the last paragraph show that there are probably two kinds of chromatic material present in the nucleus, one of which is able to pass out into the cytoplasm and one which is not able to do so. Judging from the varying amounts of chromatic material which are present in different cells of the same kind when subjected to similar conditions it seems that the non-diffusable chromatin may, when conditions warrant, change chemically so as to become able to pass through the nuclear membrane. When moisture is again available the reverse process could take place and new chromatic material be built up from cytoplasmic substance. This certainly would fit in with the changes which one is able to observe in the different stages under the microscope.

The changes in the chromatin just mentioned are not unlike those which Heidenhain ('94) has described. His oxychromatin and basichromatin could be demonstrated by staining reactions. In the rotifer cells, however, the staining is not nearly so precise and delicate in the dried tissues so that it would be difficult to say whether or not the chromatin exists in the condition just mentioned. Certain it is, however, that there is a change in the chemical nature of some of the chromatin at the time of drying and a return to normal conditions when moisture is added·

D. *Animals Recovering from Desiccation.*—The condition of the organs in rotifers recovering from desiccation is shown in Fig. 15, Plate IV. This section was made from an animal which after drying thoroughly had been put into water and then killed four hours subsequent to the addition of water. This perhaps represents a case where recovery was slower than usual, but the condition of the tissues shows that it certainly would have recovered completely.

In this same section the vitellaria are seen to be resuming their normal condition. The cytoplasm is not different from that of the normal tissue. One of the nuclei has completely recovered while the other two which appear in the section are rapidly assuming typical structure. One of the first changes noticeable in the nuclei of cells of dried animals subjected to moisture is the increase in thickness in the chromatic ring in the nucleus and the greater affinity for stains exhibited by it. This is well shown in these vitellarium nuclei. The two conditions of the nuclei shown are therefore two stages in the process of recovery. Fig. 23 represents a longitudinal section through the ovary-vitellarium of an animal which had been kept in an evacuated desiccator for fifteen days, then placed in water and killed one hour and fifteen minutes after the addition of water. The recovery of this animal was more rapid than was that of the one represented in Fig. 15 for it had already partially unfolded at the time it was killed. The nuclei here show several chromatic patterns not figured in the other sections but they are all stages incidental to the resumption of the typical nuclear form.

Fig. 22 shows a transverse section of the vitellarium of an animal dried at room temperature, then placed in an evacuated desiccator for six days, then in water for one hour and finally killed and sectioned. The nuclei here show almost complete recovery, while the cytoplasmic part of the structure has not regained the characteristic regular pattern.

Another condition found frequently in the cytoplasmic portion of the vitellaria of rotifers recovering from desiccation is that represented in Fig. 20. The vitellarium nuclei are found in various stages of recovery, while entirely outside of the nuclear membrane and indistinguishable in appearance from ovarian nuclei are round chromatic particles. This condition is important for I believe it shows what we have not been able to demonstrate visually before, namely, that the withdrawal of water causes the chromatin in the vitellarium nucleus to diffuse into the cytoplasm, but the very withdrawal of water initiates a chemical change which causes the chromatin to lose its staining power. The addition of water causes this same chromatic material, scattered about through the cytoplasm to resume its normal staining reaction and in that way its actual presence is for the first time visually demonstrated. Gradually this extranuclear chromatin in the vitellarium disappears and it is probable that its disappearance is due to some chemical change which again causes it to assume the appearance or become a part of the regular cytoplasmic structure.

In Fig. 23 the cytoplasm of the ovary shows that it has regained its clear, homogeneous appearance. The number of ovarian nuclei is also greater than in sections of that organ which have not been recently dried. I have found this condition repeatedly in sections of animals recovering from drying. This is clearly one of the steps incident to increased reproductive activity. There are several factors involved in this process. It will be recalled that in drying the cytoplasm of the ovary becomes dense and takes chromatid stains more readily than do sections of the ovary of animals in the free-swimming condition. Since, as we have seen, chromatic material passes from the nuclei of the vitellarium and of other tissues into the cytoplasm of the same structures it is not improbable that a similar movement would occur in the ovary. In the latter case, however, the elements are so small that one detects minute changes with difficulty even under the highest powers of the microscope.

This condition in the ovary of the recovering rotifer seems to indicate the time and manner of the increase of the ovarian nuclei.

It is well known that periods of great reproductive activity always follow periods of desiccation. Reproductive activity under normal conditions in most animals would involve an increase in the number of the sex cells. Now in the rotifer this increase might be looked for just before drying, for, if the cells of the other tissues prepare themselves for the new conditions by rearranging their elements it is not inconceivable that the sexual elements might likewise prepare for a season of unusual activity. Such, however, is not the case. By counting the nuclei in several hundreds of sections of ovaries in normal, dried, and recovering animals, I find that the average number in the first two conditions is about the same. Likewise in sections of ovaries of animals to which water had just been added there is no noticeable increase. But in sections of animals killed several hours after the addition of water one is struck by the increase in the number of the ovarian nuclei.

It would be desirable to make a definite statement as to the exact time which must elapse before the multiplication takes place but this is impossible since the time varies among different individuals. In animals dried several weeks the increase does not become apparent in any case before one and one half to two hours has elapsed subsequent to adding water to the dried animals. This time may be too short for animals whose processes go on at a much slower rate.

In connection with the observation regarding the position of chromatic bodies in the cytoplasm of the vitellarium it is interesting to note that Janson ('93) comments upon, but gives no figures of similar cases. He says: "Only twice in *P. roseola* and *Callidina* I saw the nuclei of the ovary surrounding the large nuclei of the vitellarium but I could not distinguish whether the peculiarity was connected with egg formation or was due to pressure." In this connection I would call attention to the position of three small nuclei in the section of the vitellarium of the rotifer recovering from desiccation shown in Fig. 15. This is one of the many cases I have observed where small numbers of these nuclei are found in the vitellarium. I am certain from observations on hundreds of similar sections that this condition is due to normal causes and cannot be laid to accidental pressure as Janson suggests. I have not been able to follow all the stages in the early development of the egg, but I believe that this condition is a stage in the development of the egg and is not to be confused with those cases where chromatin arising from the nucleus of the vitellarium is found in the cytoplasm of that organ.

The condition of the different gland cells in animals recovering from desiccation is shown in Figs. 15, 18*b* and 26. Fig. 18*b* is a section of foot gland cells of a rotifer which was kept for fourteen days in an evacuated desiccator and then placed in water one and one fourth hours previous to killing. The nuclei are seen to be rapidly assuming the typical condition which is represented in Fig. 18*a*. The cytoplasmic changes in these glands are perhaps less marked than in any of the other tissues considered. This may be due in part to the nature of their contents, for being impregnated with their gelatinous secretion they would show fewer changes than would other cells whose plasma is less viscous.

The salivary glands in animals recovering from desiccation show the presence of recently acquired water by the vacuoles scattered at regular intervals throughout their cytoplasm. As recovery becomes complete the cytoplasm assumes the condition shown in Fig. 24 and a typical half-recovered condition is well shown in Fig. 26. In this figure it will be noticed that the karyosomes in the nuclei are of smaller size than in the normal tissue. They have resumed the normal condition, however, in all respects except size.

In the stomach tissue of the rotifer recovering from desiccation the absence of the food granules is again noticed. The objection might have been offered in the case of the dried stomach that the failure of the food granules to become apparent was due not to their actual absence but to a chemical change incidental to the drying process which made them entirely lose their affinity for stains. If this were the case it seems that upon the addition of water the granules would again resume their normal staining reaction. That this possibility is not realized in fact is apparent from an examination of the stomach tissue shown in Fig. 15. The nuclei have resumed their normal appearance. The cytoplasmic syncytium of the stomach tube compares favorably with that in the normal animal, while scattered about through it are vacuoles which probably mark the previous positions of the food particles which have disappeared. In any event, the food particles have been used up and the stomach tissue has the same appearance as is found in cases where the animal is not dried, but starved.

The trochal cilia in animals recovering from desiccation under the highest magnification cannot be seen to be different from those in the normal or even in the dried animal. Absolutely no change is observable.

Upon the addition of water the hypodermal layer of the integument rapidly swells and assumes its normal thickness. It might be mentioned here again that if there were any sign of a protecting gelatinous capsule it should be observed at this point when the different tissues swell and draw apart. But here, as before, there is absolutely no sign of such a gelatinous envelope.

The cells of the peripheral layer of the brain of rotifers recovering from desiccation show the characteristic chromatic ring in their nuclei (Fig. 29, Pl. V.). This, at first sight, is remarkable because the nuclei of similar cells in the dried animals do not particularly show such a disposition of the chromatic material. However, the cells in the peripheral layer of the brain are among the smallest in the rotifer body and their nuclei are correspondingly minute. The small amount of chromatin in each nucleus could migrate toward the nuclear membrane without being especially apparent. But, as was pointed out in an earlier paragraph, the addition of water causes a swelling together with an increase in staining capacity in the nuclear material with the result that the position of the chromatin is most plainly seen not in the dry period but just subsequent to that and before a sufficient time has elapsed to permit a normal arrangement to be brought about.

The observations upon the tissues of the dried animals recorded in the preceding paragraphs show that they react similarly, with very few exceptions, to the drying stimulus. The cytoplasm becomes denser. This is of course to be expected. The nuclei, all of which have a very definite and similar chromatic pattern, undergo a chromatic rearrangement which is striking and uniform. That this chromatic movement is the visible expression of an adaptation to a new sort of environment can hardly be doubted. The resumption of normal conditions in nucleus and cytoplasm is likewise uniform in the elements of the different tissues. If any doubt existed as to the nature of the adaptive response in the desiccated condition that uncertainty would be removed upon observing the return to normal conditions as shown in sections of recovering rotifers. The significance of the chromatic movement and its interaction with the cytoplasm in the different tissues will be discussed fully in a later section.

IV. Review and Discussion.

The literature relating to the cytology of desiccation is, in the case of animals, very sparse, and, relating to rotifers, almost nothing. While rotifers have been studied since the invention of the microscope, the chief interest has been along anatomical lines. Along with the anatomical studies, the Bdelloideæ have received consideration in discussions as to whether they actually dry up and recover from the desiccation, but apparently no one thought to examine the cytological changes underlying the process.

Pfeffer, in his "Physiology of Plants," makes numerous observations upon the subject of desiccation among members of the plant kingdom. His point of view is, of course, physiological rather than cytological.

Pfeffer suggests that where death occurs as a result of drying it may be due in part to the removal of the last traces of absorbed or combined water. He says: "Since full turgor is restored in mosses and other plants immediately after moistening them, it is evident that the osmotic materials remain as solids in the central vacuole. Evidently, therefore, the protoplasm is not injured by the concentrated cell sap. If, however, the latter is responsible for the death of certain plants on drying, we have revealed to us in such cases the immediate cause of the fatal action of desiccation. It is, however, hardly likely that the death of plants killed by the removal of more or less of the imbibed water, as well as that of those only killed by the removal of the last traces of absorbed or even combined water, is alike produced in the same way."

I believe that the work of McDougal, Long and Brown, mentioned in the next paragraph shows that plants may have their cell sap concentrated in the fashion described by Pfeffer and still recover from the process. My own observations upon *Philodina* lead me to think that death at the time of drying cannot be attributed to such a cause but rather to a lack of a complete adaptive rearrangement of cytoplasmic and nuclear material.

Among the botanists the subject of desiccation is now being studied by McDougal and his associates at the Desert Laboratory at Tucson, Ariz. A recent paper from that laboratory deals with cytological phenomena in the desiccation of *Echinocactus*. J. G. Brown, who did the cytological part of the work, examined four kinds of cells in the tissues of *Echinocactus wislizeni*, viz., integument, palisade, outer cortex and deep cortex. Starting with a description of these cells in sections of a plant grown under normal conditions he compares similar cells from sections of (1) a plant which had been desiccated six years; (2) one which had been desiccated ten months; and (3) one which had been desiccated forty-two months and then allowed to grow under normal conditions for twenty-two months.

In the palisade cells the results of desiccation were most apparent. These cells are rectangular in section, having a peripheral layer of protoplasm embedded in which are the nucleus and plastids. A corresponding cell from a plant desiccated six years showed the cytoplasm gathered in one corner of the cell. The nucleus, which normally is of lenticular form and contains vacuoles, was still embedded in the cytoplasmic mass. It had decreased to one fourth or less of the size of the normal nucleus. The vacuoles had disappeared and the chromatic elements had for the most part become aggregated in a ring in the region of the nuclear wall.

In the palisade cells of a cactus which had been desiccated only ten months the cytoplasm did not deviate from the normal condition as in the first case mentioned, at least in so far as its position was concerned. The nuclei decreased almost as much in size and assumed practically the same form as those in the palisade cells desiccated six years. In the specimen which was desiccated forty-two months and then returned to normal conditions for twenty-two months, the cytoplasm was found to be normal as to quantity and position, while the nuclei, although increased slightly in diameter, still retained the ring form characteristic of the dried condition.

In the other tissues the results were quite similar to those recorded for the palisade cells. In the integument the cuticle thickened slightly in the specimen desiccated six years. The outer epidermal cells were thinner in the plant dried six years than in that dried only ten months, while in the one desiccated forty-two months and returned to normal surroundings for twenty-two months the epidermal walls were of about the same thickness as in the normal tissue.

The nuclei of the epidermal cells decreased to about one half normal size in *Echinocactus* No. 7. The characteristic peripheral chromatic ring appeared. In specimen No. 6 which after desiccation was returned to a normal environment, the epidermal nuclei résumed their normal condition.

In the cells of the outer cortex below the palisade layer, the cell walls increased perceptibly in thickness as desiccation proceeded and they recovered somewhat their normal thickness as moisture was again admitted. The nuclei shrunk to about half size and assumed the ring form. In the deeper cortex practically the same things happened as were just described for the outer cortex. The cytoplasm decreased almost to the point of disappearance in the cactus dried six years and gradually increased in volume in the plants subjected to less severe conditions. The nuclei decreased to about half size. The cell-walls thickened enormously in the extreme cases of drying and in many cases never recovered normal thickness.

The results of McDougal and Brown are most interesting when compared with my own. The observations upon the cytoplasmic and nuclear changes in the dried tissues show that they are similar in many respects with those in corn embryos and rotifer tissues described in my earlier paper.

The length of time during which an *Echinocactus* may survive in the open at the expense of its surplus food material and water was found to be no more than two years while similar plants in diffuse light were sound after six years of starvation. The cactus, then, which normally lives in an environment containing little moisture is less able to adapt itself to extreme dry conditions than is the rotifer which normally swims about in water. The extreme length of time during which a *Philodina* may remain dry is not known but well authenticated records show that they have been known to withstand a period of dryness for as much as twenty-seven years. To date no experimental data are recorded to show the effects of varying intensities of light upon the time during which rotifers may live in a desiccated condition. This point I hope to treat in a future publication.

Another result of interest in the cactus experiments is that in extended desiccation and starvation the plasmatic colloids are eventually broken down by katabolic action. This katabolic activity includes hydrolysis of the cell-walls of the cortex. Now in *Philodina* katabolic activity undoubtedly takes place during the dry periods. This was most clearly seen in the tissue of the stomach-intestine. But while the reserve food granules disappear, no change is observable in the walls of any cell. It might be said from the nature of the two cases that the reaction in the cactus is irreversible while in the rotifer it is reversible, for the cells of the cactus perhaps never assume a perfectly normal condition after the drying process while a sufficient food supply is all that is necessary to make the rotifer quickly resume its normal structure.

McDougal found that in the cactus the loss in weight in full illumination may not greatly exceed 50 per cent. of the water present without producing death by desiccation. Now under normal conditions about 95 per cent. of the weight of the cactus is made up of water. This would mean that a decrease of approximately one half in the weight of the plant as a result of drying, is usually fatal. In *Philodina* the minuteness of the animal, together with the fact that it normally lives in water, makes it almost impossible to determine the changes in weight during the drying period. It is easy, however, to follow the changes in volume which occur during drying and anyone can see if he will dry a *Philodina* upon a slide under a microscope that the animal decreases to from one third to one fourth of its original size. Since most of the weight of the rotifer body, certainly more than 95 per cent., is made up of water it will be seen at once that the percentage of substance lost by the rotifer during desiccation is much higher than that observed in the cactus.

The desiccation process in the cactus caused few changes in the thickness of cell-walls. In the plant treated for seventy-three months the cuticle was slightly thicker than normal while the outer walls of the epidermal cells were thinner. This result was obtained in the most severe test of the series. In my experiments on *Philodina* the most severe drying caused absolutely no thickening of the cell walls which bordered the surface, but on the contrary by a loss of water from the hypodermal layer an actual decrease in the thickness of the integument was observed in all cases.

Perhaps the most pronounced cytological effects of desiccation are those recorded for the cortex cells of *Echinocactus*. Here there was an entire disappearance of the protoplasts and a hydrolysis of the cell walls. There is nothing in my experiments upon *Philodina* which parallels this. The changes described in the stomach tissue are similar but in no case have I ever observed the disappearance of cell-walls or the formation of spaces as a result of the disintegration of cell-elements.

That the different changes during the desiccation process and subsequent recovery in *Echinocactus* are very slow is well shown by McDougal and Brown when they state that a plant which had been desiccated for forty-two months and was then placed under normal conditions in the soil for twenty-two months did not entirely regain the normal condition. It would be interesting to know if the plant would have recovered if given more time for the process. In *Philodina* the process of recovery begins upon the addition of water and is complete in a short time, never longer than a few hours.

For an analysis of the meaning of these changes I should say that the cactus is unfavorable for rapid results. The great length of time necessary for changes to take place and the slowness with which normal conditions are resumed makes experimental work with a large number of specimens difficult. This probably accounts in part for the fact that McDougal and Brown make no attempt to suggest the significance of the cytological changes which they describe.

Metabolic Water and Its Relation to Desiccation.

For a complete discussion of the subject of metabolic water and its functions in an organism I would refer the reader to the excellent paper of Babcock ('12). For the present purpose it will be sufficient to outline the main points of the subject and relate them to the material under discussion.

Desiccation is the operation of drying or removing water from a substance. When the last trace of water is removed the desiccation is said to be absolute. Absolute desiccation of chemical crystals is brought about by heating the substance to dryness or when the material involved is readily decomposed by heat the operation is accomplished by the use of a desiccator. A condition of absolute desiccation can be maintained so long as the heat is applied in the one case or so long as the substance is kept within the drying chamber in the other. When the desiccating influence is removed moisture is absorbed.

Inorganic crystals can, as a rule, be deprived of their moisture only by a temperature of 100 degrees centigrade or higher. Not only are the crystals as such made dry but the water of crystallization, by virtue of which the crystals exist, is driven off and the substance assumes an amorphous condition.

Living organisms contain water combined with them in a fashion which for present purposes may be compared to water of crystallization. In order to remove all water by means of heat it is necessary to raise the temperature of the organic material in question until actual charring takes place. It is evident, therefore, that the absolute desiccation of living substance cannot be accomplished by means of heat without destroying life. On the other hand, the second method of desiccation mentioned above will not remove the last trace of chemically combined water from living tissue. Since an absolute desiccation of living things without the destruction of life is impossible by the second method it seems certain that no rotifer or other living thing has ever lived after an absolute desiccation.

The recorded observations upon the desiccation of living things, whether animal or plant, have to do with a relative rather than with an absolute desiccation. If the water content is lowered much below the normal percentage the organism may be said to have undergone an actual or even a complete desiccation but it will be understood that in all cases this does not imply that the condition of dryness is absolute.

Water may be acquired by an organism in three ways: (1) It may be imbibed directly; (2) it may be taken in with solid food, and (3) it may be formed within the organism by metabolic changes in the organic constituents of the food and tissues induced by respiration and other vital processes.

Imbibed water probably makes up the greater part of the cell-sap. Its method of combination with the living substance may be, as Nägeli contended, not chemical but a mere physical attraction due to minute molecular aggregations designated as micellæ, between which the water enters by capillary attraction and forcing the micellæ apart increases the volume of the tissues. On the other hand, as Babcock points out, all of the phenomena of imbibition point directly to a molecular combination between the substance composing an organized body and water. The combination is in most cases feeble, since it is broken up by a relatively low temperature without changing the molecular structure of either the solid tissue or the water. It is however analogous to the behavior of many substances, both organic and inorganic, which crystallize with water of crystallization.

The water taken in with solid food probably has in part the same fate as imbibed water although its history is slightly different. In *Philodina* the distinction between these two sources of water is not marked and the same can be said of any animal having a similar habitat and feeding habits.

The production of metabolic water, in certain stages of the life-histories of both plants and animals is sufficient for all purposes for considerable periods of time. In the resting periods of deciduous plants, in bulbs, in tubers and especially in seeds and spores, ample water is provided for all vital processes by the slow oxidation that takes place as a result of direct respiration. Hibernating animals receive no water from external sources for several months, although water is being constantly lost by respiration and excretion. Many varieties of insects such as clothes moths, grain weevils, dry-wood borers, etc., are able to subsist during all stages of development upon air-dried food materials containing less than 10 per cent. of water; in these cases nearly all the water required is metabolic.

Metabolic water may be formed as a result of two kinds of respiration. In *direct respiration* the organic matter comprising the food and tissues of an organism is oxidized by means of free oxygen derived from the air during respiration. Many organisms when deprived of free oxygen are capable of maintaining for a short time certain of the respiratory functions, and deriving energy from food material and from tissues by breaking up the molecular structure into new forms of a lower order. This is known as *intramolecular respiration*.

When a *Philodina* dries it loses all its free water. The spaces between the different organs are filled during the free-swimming existence of the animal, with fluid. This fluid between organs is the first to disappear. The loss of uncombined water is responsible in large measure for the decrease in size of the animal during the drying process. By examining Figs. 1 and 2 it will be seen that in their normal condition the organs are not closely packed together. It is these spaces between organs which allow the animal to decrease so appreciably in size and in such a decrease the fluid content of the spaces is lost.

In addition to the loss of free or uncombined water there is a corresponding though not extensive loss of combined or chemically bound water. This is the imbibed water mentioned in a previous paragraph. Just as water is distributed in the interstices of a sponge, so water is held in every cell of the rotifer body. It is hardly necessary to emphasize again in this connection that such water is probably not held by capillary attraction as in a sponge but by a loose molecular union. The presence of this water in the rotifer tissues is demonstrable in a visual fashion, for in the sections of normal undried rotifers the cells all possess vacuoles between the granules or reticulum of the cytoplasm. These vacuoles or spaces are certainly filled with the more fluid cytoplasmic ingredients and their true fluid nature is demonstrated upon examination of a section of a dried animal where they have with few exceptions disappeared, or better still in an animal recovering from desiccation where the former position of granules of reserve food material is marked by vacuoles undoubtedly containing liquid. In a previous paragraph attention was called to the fact that in those cells whose cytoplasm is loosely granular in the normal condition, the granules were closely packed in similar cells of a dried animal. The change in space relation of these cytoplasmic granules is due to loss of imbibed water.

The amount of imbibed water which an organism may lose and still live varies with the kind of organism. Seeds which in a dormant condition contain from ten to not more than twenty per cent. of water may still retain their viability and germinating power if more than half of the water content is removed. This viability of corn with different degrees of moisture content is recorded by Babcock ('12) and I have repeated many of his experiments and find them to be correct.

In McDougal's cactus experiments it was found, as was before mentioned, that loss of weight in full illumination may not greatly exceed fifty per cent. of the water present without producing death by desiccation. In seeds, on the other hand, a loss of seventy-five per cent. of the water content need not necessarily be fatal and in some cases certainly the percentage loss may be higher without death resulting.

In *Philodina*, while exact quantitative measurements are exceedingly difficult, the water loss judged by decrease in size of the drying animal and by the nature of the dried tissues, must certainly be higher than that of seeds.

The Mechanism of Metabolism in the Dried Rotifer.

Admitting that the desiccation of *Philodina* is complete and admitting further that metabolism takes place though much retarded, in the dried condition, the mechanics of the metabolic activity is the next point to demand explanation.

It will be remembered that Jacobs ('09) showed by means of intra vitam staining with chemical indicators and subsequent tests with various gases, that the integument of the dried rotifer is at all times freely permeable to gases. Attention was called, in another paragraph of this paper, to the fact that many animals when deprived of their normal supply of moisture could still exist for a time by means of the direct action of oxygen from the air upon the complex materials of the tissues or upon inclusions of complex food materials within the latter. This action would of course not be sufficient to prolong life indefinitely for the time would come when all the available reserve food material would be exhausted or the accumulation of poisonous waste products might end life. That such a state of affairs might be realized in the case of a dried *Philodina* in the air is not impossible. Certain it is that this animal can live a long time; many years in fact, in a dried condition without food from external sources. Its lease of life, however, under these conditions, is not indefinite as is evidenced by the fact that under the most careful conditions of drying some of the rotifers always die. To explain these fatal cases it would seem that the store of reserve material became exhausted or the metabolic products accumulated in too great a quantity and death was the result. It seems reasonable to assume then, that under the conditions just outlined metabolic activity goes on through oxidations of complex substances within the tissues of the animal by means of oxygen obtained by direct respiration.

Those rotifers which were described as having been kept in an evacuated desiccator for varying periods and which survived the experiment could not have their metabolic activities explained upon the same basis as the ones which were dried in air. It will be recalled that mention was made of certain cases where metabolism might proceed in the absence of air by means of intra-molecular activity. In these cases the complex substances which are an integral part of, or are merely inclusions in the tissues have their molecular structure broken up into newer forms of a lower order. In this way energy is derived from food material and life may be prolonged for a greater or less period of time depending upon the amount of reserve material present.

Both types of chemical reactions outlined above are hydrolytic processes. This means then that metabolic water is being evolved in small quantities during the entire dry period.

It should be understood here that the processes just outlined are hardly possible of actual demonstration for if they are sufficient to keep such a small organism as a rotifer living for years in the dried condition they must of necessity be exceedingly slow. Observations made by Babcock ('12) show that respiration and consequent oxidations in seeds and spores are practically suspended, it being possible to detect them only by observations extending over long periods of time. If this be true of an embryonic structure with its simple organization it is true to a greater degree in an adult animal in a state of retarded activity.

Death ensues in all dormant organisms at the end of a certain time. The period of dormancy is limited. In *Philodina*, since metabolism proceeds continuously but slowly as I have shown, the death of an animal can be attributed to starvation rather than dryness. Dryness is a contributing cause of death but the animal dies as a result of lack of food rather than as a result of lack of moisture.

We may briefly summarize the previously mentioned causes of death in the dried rotifer as follows: (1) Mechanical injury due to too rapid drying, (2) starvation resulting from lack of reserve food material, (3) poisonous effect of metabolic products and (4) insufficient time before drying to effect the nuclear-cytoplasmic reorganization.

The Significance of the Nuclear-Cytoplasmic Interchange During Desiccation.

The nucleus in the cells of most of the tissues of *P. roseola* consists, as was pointed out before, of a single large karyosome surrounded by a clear area and having for its boundary a definite nuclear membrane. This karyosome is of course not a true nucleolus since it is made up of chromatic material. This type of nucleus has been given the name of "nucleoles noyau" by Carnoy and it has been described by numerous authors in the cells of several kinds of unicellular animals. Nucleoli showing apparently all grades of morphological and chemical properties between true plasmasomes and karyosomes have been described by various authors (see Montgomery, '98) so that it cannot be said that there are no intermediate conditions to be found. Montgomery says: "The existence of Carnoy's 'nucleoles mixtes' and 'nucleoles-noyaux' in cells of metazoa appears to be doubtful" yet the "pseudonucleoli" which he describes in the ova of the mollusc *Montagua pilata* have many of the properties of a chromatin nucleolus or karyosome.

The significance of the peripheral chromatic ring in the dried rotifer nucleus is a point which requires explanation and correlation with the other conditions incident to the adaptation of the rotifer to its dried condition. The chromatin ring undoubtedly represents a stage of chromatin migration. There are two possibilities regarding the extent of this migration. The chromatin may merely leave its place in the center of the nucleus and, by taking a position next to the nuclear membrane, remain within the nucleus proper during the entire dry period. On the other hand, some of the chromatic elements might be of such nature as to pass readily through the nuclear membrane while the latter might be impermeable for others. That materials may be changed chemically within the nucleus is admitted. In nuclei of cells which have just completed mitotic division the nucleus imbibes substances from the cytoplasm and changes them into nuclear material. It is also a matter of common knowledge that at the beginning of mitotic activity much material is cast out of the nucleus into the cytoplasm. However, it is not commonly contended that materials pass from the nucleus into the cytoplasm without a rupture of the nuclear membrane. That the latter condition is a possibility in the rotifer nucleus will appear shortly.

The karyosome of the rotifer nucleus being almost pure chromatin, the reason for its migration might be sought in a consideration of some of the functions of chromatin in general. R. Lillie ('02) and others have shown "that in many tissues the nucleus is the chief agency in the intracellular activation of oxygen; and further that the active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm." Now the parts of the nucleus which take part in these oxidations must be the chromatin and, perhaps, the nuclear membrane. It seems reasonable to suppose that the lack of the normal amount of water in the nucleus causes the chromatin to be unable to interact with the nuclear membrane in bringing about oxidations, and the nucleus adapts itself to the new conditions by rearranging its chromatic content in the manner described.

I have implied in the preceding paragraph that the chromatic material goes no further than the nuclear membrane and that there it is deposited to make the typical ring structure. While this may be and probably is true in cases of relatively incomplete desiccation, I think there is much evidence that in many cases the chromatin either does pass through the nuclear membrane or it is so changed chemically within the membrane that its presence cannot be detected by ordinary methods. A comparison of different nuclei in the figures of sections of dried animals shows that the peripheral chromatic ring is not nearly uniform in thickness in the different nuclei. I interpret this as meaning that varying amounts of chromatic material have been able to pass to the cytoplasm,—the ability or inability to do so depending perhaps for one thing upon the permeability of the nuclear membrane.

It will be remembered in this connection that immediately upon the addition of water to the dried animals the chromatic ring thickens and regains its normal staining power. This fact also is, I think, capable of two interpretations. It may indicate that chromatic material which moved in some form into the cytoplasm during the dry period immediately begins to return to its normal position when moisture is restored or it may be that the drying process caused the chromatin to lose its staining power to such an extent that its volume only seemed to be diminished. From the appearance of the sections I do not think that there is any doubt but that the former supposition is correct. The chromatic material in some form actually does pass into the cytoplasm during the dry period.

Whatever may actually happen to the chromatin in the way of a change of position it seems certain that the purpose of all the position changes outlined are one, namely to keep the chromatin within a working radius of the material to be oxidized. The oxidations referred to in this connection are the breaking down of complex food materials in the cytoplasm with the consequent release of metabolic water during the process. The details of this process are discussed in another paragraph.

The nucleus of the gregarines is similar in many points of structure with that of the rotifer. Montgomery ('98), speaking of an unnamed gregarine from *Carinella annulata* says: "Now as the gregarine grows, at the same time both nucleus and the total mass of the nucleolar substance increase in size; but the nucleus cannot grow without the addition of a substance or substances to it which have been derived from without. Accordingly, I suppose that the substance of these granules has an extranuclear origin, a substance, *i. e.*, which having penetrated the nucleus from the cytoplasm, undergoes a chemical change in the nucleus and there becomes precipitated in the form of granules, for no such substance occurs in granular form in the cytoplasm. The growth of the nucleoli might then be explained on the assumption of the intussusception of this substance by the nucleoli." It seems probable that the rotifer nucleolus grows at the time of recovery from desiccation in the fashion outlined by Montgomery for the gregarine nucleolus. Drying then, brings about a reversal of this process and the normal condition is resumed only when moisture is restored.

In considering the movements of chromatin from the nucleus into the cytoplasm one is struck by the similarity between the phenomena here observed and those described by Woodruff and Erdmann in their paper upon periodic reorganization in *Paramœcium aurelia*.

These authors working upon Woodruff's non-conjugating line of *P. aurelia*, found that there was a periodic nuclear reorganization. "This nuclear reorganization consists of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously a multiplication of the micro-nuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one or two form the new macronuclear and micronuclear apparatus. This results in the reorganization of the cell without the fusion of two animals." They find not only that the reorganization process is coincident with the low point between rhythms but also that there is a causal relation between the reorganization process and the rhythms. Woodruff defines a rhythm as a minor periodic rise and fall in the division rate from which recovery is autonomous. Woodruff believes that the rhythmical changes which he describes are inherent in the phenomena of the cell. There are changes in *Paramœcium*, described by Calkins and others, involving a somewhat similar reorganization of the cell but whose cause may be laid in part to environmental conditions.

The life history of *Philodina* consists in periodic increases and decreases in activity as a result of environmental conditions and in many respects its structural responses are similar to those described by Woodruff and Erdmann in *Paramœcium*. The environmental stimulus acting upon the rotifer to produce the periodicity is dryness. The response to the stimulus is an interaction between nucleus and cytoplasm not unlike that taking place in *Paramœcium*. Normal conditions of organization are resumed as soon as the stimulus is removed and the noticeable result of the entire process is an increase in reproductive activity. Of course many of the physiological processes of the two animals are markedly different in many respects and would not admit of close comparison but this one particular phase in which there is such a marked agreement in the cytological phenomena accompanying a physiological state seems worthy of comment.

The Relation between Desiccation and Reproductive Activity.

It is hardly necessary to again call to mind the causal relation between desiccation and reproduction in *Philodina*. The numerous observations of many authors has established this as a fact. It is, however, profitable to speculate concerning the specific cause of the increased reproduction. It is insufficient to say that drying is the cause. It is necessary to point out if possible how desiccation causes the reproductive increase.

In an earlier paragraph I have shown that the nuclear-cytoplasmic reorganization takes place in the ovary the same as in other tissues, and that just subsequent to the addition of water an increase in the number of ovarian nuclei takes place. I have not been able to observe these elements in the act of multiplication but I am sure concerning the results of the process if not of the method.

It seems, then, that we are dealing with an adaptive, structural response of a special sort. Whereas in the other tissues the reorganization of cell elements took place to satisfy a vegetative or metabolic demand, here we have a similar reorganization, the end of which is increased reproduction, and the stimulus to which is in both cases,—desiccation.

In addition to the increase in the number of sexual elements, a stimulus is also apparently given which leads to the immediate development of some of these elements.

That egg cells should be stimulated to develop as a result of loss of water is neither a strange idea nor a new one. Loeb ('06) in his experiments upon artificial parthenogenesis found that the unfertilized eggs of *Arbacia* and *Strongylocentrotus* could be induced to develop into swimming larvæ by immersing them in hypertonic sea-water and later placing them in water of normal concentration. That the initiation of development was due to the withdrawal of water he demonstrated by further experiments in which the eggs were not put back into sea-water of normal concentration. In this case only a loss but no taking up of the water occurred, yet swimming larvæ developed.

Philodina is a parthenogenetic rotifer, and as has been shown is stimulated to reproduce by removal of moisture from its tissues and consequently from the sex cells. It would seem, therefore, that we are here dealing with a process which is natural and commonly employed by this parthenogenetic animal as a result of environmental conditions but that the same stimulus can be employed to bring about parthenogenetic development among an entirely different group of animals which reproduce normally by the sexual method. The steps in the two processes are not to be easily compared, yet the initial stimulus is similar and development is the result in both cases.

Desiccation Phenomena in Their Relation to the Subject of Adaptation.

That desiccation is but one of the unfavorable conditions to which rotifers may adapt themselves is evident from the literature. The Bdelloideæ can easily adapt themselves in other ways. Murray in his account of rotifer fauna observed during the first Shackleton south polar expedition found that *Callidina constricta* and *Adineta grandis*, both *Bdelloids*, were able to live for rather long periods in salt water although normally they are found only in fresh water. Murray in the account of his experiments says: "To test the degrees of cold which they could stand blocks of ice were cut from the lakes and exposed to the air in the coldest weather of the whole winter. By boring into the center of the blocks we found that they were as cold as the air. A temperature of − 40° F. did not kill the animals.

"Then they were alternately frozen and thawed weekly for a long period and took no harm. They were dried and frozen and thawed and moistened and still they lived. At last they were dried and the bottle containing them was immersed in boiling water, which was allowed to cool gradually and still a great many survived. Again they were put into sea water and into the brine from the bottom of Green Lake which is so salt that it only freezes at about 0° F. They were kept in these salt waters for a month, yet as soon as they were transferred to fresh water they began to crawl about as though nothing had happened.

"Such is the vitality of these little animals that they can endure being taken from ice at a minus temperature, thawed, dried and subjected to a temperature not very far short of the boiling point, all within a few hours (a range of more than 200° F.)."

It is desirable that the structural changes, if any, which accompany freezing and salinity of the surrounding medium be worked out in order to compare the resulting conditions with those in the dried animals. It is probable that when the different environmental conditions were brought about successively in varying combinations, the structural response is somewhat different in each case.

Bachmetjew ('07) showed that juices of the insect body do not completely congeal till they have been reduced to − 4.5° C., but at this temperature the insect does not yet die.

Reiff ('09) kept an adult *Actias selene* at a temperature of − 3° to − 6° C. from November 23 to January 3. Upon raising the temperature to 17° C. the insect again became active. The normal length of life of this species of *Actias* averages seven to eight days. The total number of days which the two insects experimented upon lived in an active condition was about two days less than the average.

Bachmetjew says that metabolism cannot take place in a frozen insect because it is impossible for the blood to circulate. However this may be, we have shown that metabolism takes place in the dried rotifer in the absence of a circulating fluid. Furthermore the experiments of Reiff just mentioned show that the total length of life is lessened in an insect when freezing intervenes. This strongly suggests that the life processes go on slowly here also. It seems desirable that the question of structural changes and metabolic processes during freezing among certain animals should be carefully investigated.

V. SUMMARY.

1. The tissues and the parts of the individual cells of a desiccated *Philodina roseola* maintain their identity during the drying process.

2. No protecting membrane is secreted when drying begins or at any other time during the desiccation process. The integument of the dried rotifer is thinner than that of the undried specimen.

3. Metabolism goes on slowly in the dry condition as is evidenced by changes in the walls of the digestive tube.

4. Desiccation in *Philodina* may be complete but not absolute without fatal results.

5. The typical nucleus in all the tissues of *Philodina* consists of a single large karyosome surrounded by a clear space with a distinct nuclear membrane at the periphery of the clear space.

6. The general effect of desiccation upon the cells of the rotifer tissues is the production of a nuclear-cytoplasmic rearrangement

in which the chromatic part of the nucleus migrates to the periphery and the cytoplasm becomes more dense.

7. The movement of chromatin during desiccation phenomena takes place in order that the cell oxidations may continue during the dried condition.

8. The extent of the structural rearrangement which takes place in the rotifer cells is not directly proportional to the intensity of the stimulus.

9. In animals recovering from desiccation the elements of nucleus and cytoplasm gradually resume normal relationships.

10. The chromatic rearrangement in the nuclei of cells of drying rotifers takes place at the very beginning of the drying process.

11. The acceleration of reproductive activity just subsequent to drying is traceable to an increase in ovarian nuclei. This increase takes place while the animal is recovering.

12. The cytological changes attending a recovery from desiccation are in their nature the exact reverse of those taking place during the drying process.

13. The death of cells as a result of desiccation is probably not a result of the activity of concentrated osmotic materials upon the protoplasm as Pfeffer suggests.

14. The death of rotifers during the desiccation process may be due to one or a combination of the following causes: (1) Mechanical injury due to too rapid drying, (2) starvation resulting from a lack of reserve food material, (3) the poisonous effect of metabolic products and (4) insufficient time before drying to effect the nuclear-cytoplasmic reorganization.

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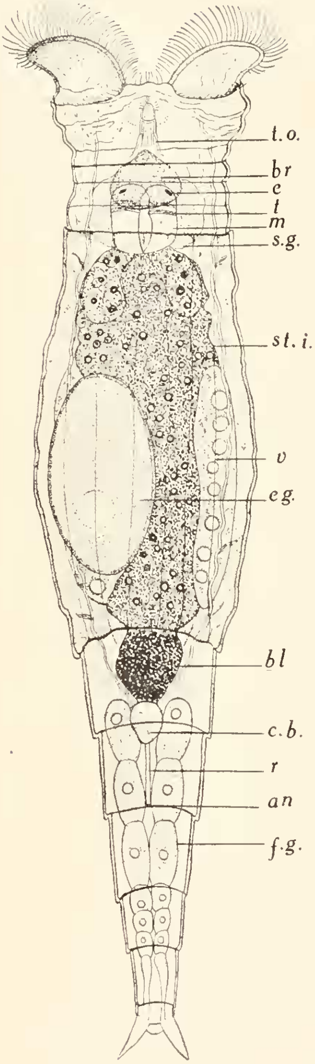
VII. EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Dorsal view of a living extended *Philodina roseola*. *t.o*, tactile organ; *br*, brain; *e*, eye; *t*, tooth; *m*, mastax; *s.g*, salivary glands; *st.i*, stomach intestine; *v*, vitellarium; *eg*, egg; *bl*, "blasendarm"; *c.b*, contractile bladder; *r*, rectum; *an*, anus; *f.g*, foot-gland.

FIG. 2. Ventral view of same animal. *ph*, pharynx; *s.g*, salivary gland; *f.c*, flame cell; *e.c*, excretory canal.



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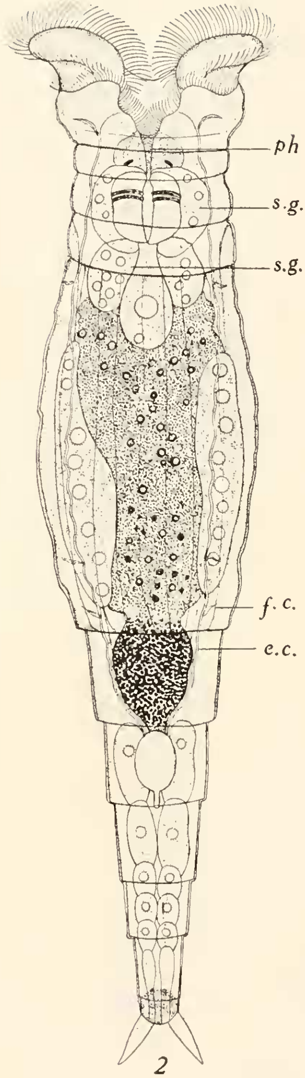

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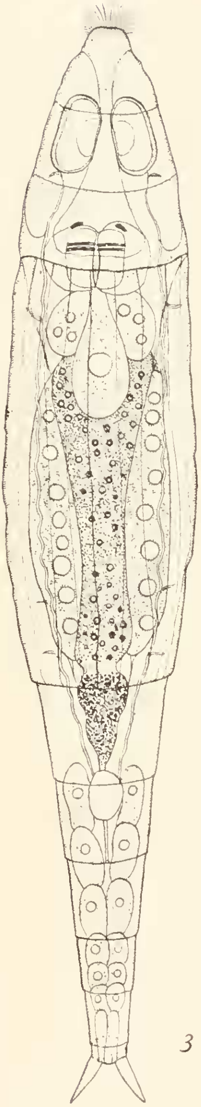
Plate II.

Fig. 3. Ventral view of *Philodina roseola* with trochal discs retracted and proboscis extended.

Fig. 4. *Philodina roseola* in contracted condition.

Fig. 5. *P. roseola* in dried contracted condition.

Fig. 6. Side view of foot of *P. roseola* showing toes.



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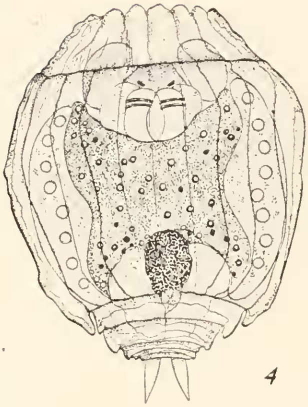

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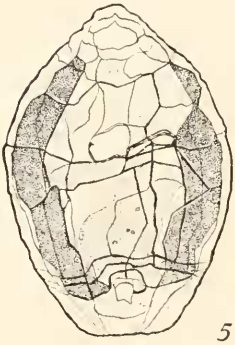

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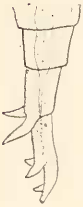

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PLATE III.

FIG. 7. Section through brain and posterior part of pharynx of normal extended animal. Zeiss. Oc. 6, Obj. 2 mm.

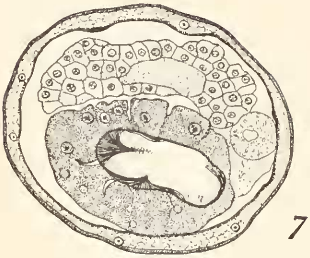
FIG. 8. Section just posterior to the mastax and through salivary glands and oesophagus. Zeiss Oc. 6, Obj. 2 mm.

FIG. 9. Section through mid-body region. Leitz Oc. 4, Obj. 2 mm.

FIG. 10. Section through mid-body region of animal containing egg. Zeiss Oc. 6, Obj. 2 mm.

FIG. 11. Section through point of juncture of stomach-intestine and "blasendarm." Zeiss Oc. 6, Obj. 2 mm.

FIG. 12. Section through contractile bladder and foot-glands. Zeiss Oc. 6, Obj. 2 mm.



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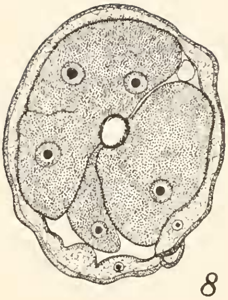

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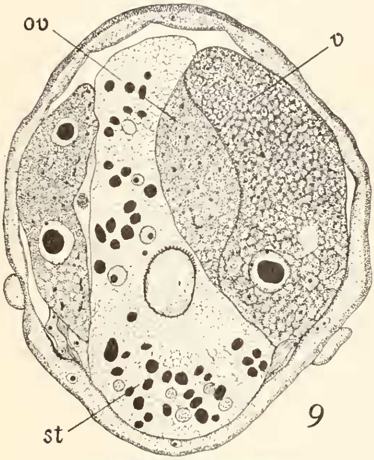

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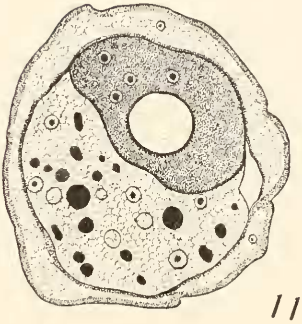

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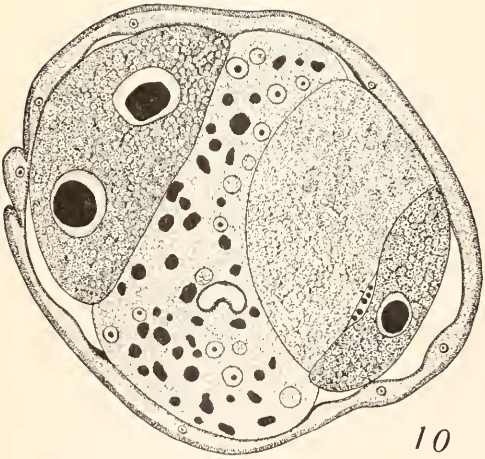

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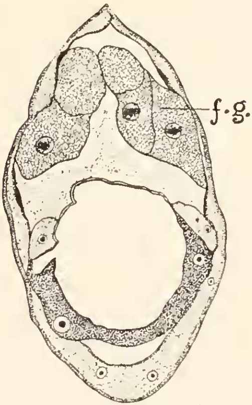

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Plate IV.

Fig. 13. Frontal section through middle of contracted *P. roseola*, not dried. Zeiss Oc. 6, Obj. 2 mm.

Fig. 14. Slightly oblique section through a contracted animal, not dried. Leitz Oc. 4, Obj. 2 mm.

Fig. 15. Frontal section through middle of a rotifer recovering from desiccation. Animal was killed four hours subsequent to the addition of water. Zeiss Oc. 6, Obj. 2 mm.

Fig. 16. Frontal section through rotifer kept in an evacuated calcium chloride desiccator for eighteen days previous to fixation. Leitz Oc. 4, Obj. 2 mm.

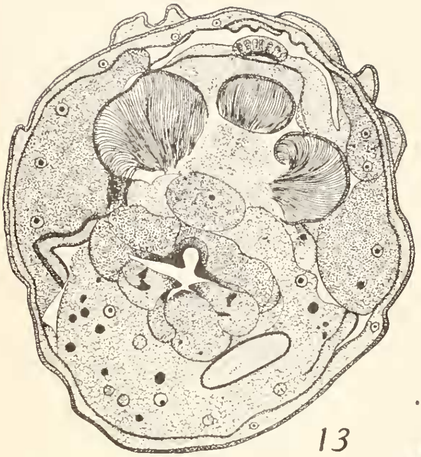
Fig. 17. Section of foot-gland cells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to time of fixation. Leitz. Oc. 12, Obj. 2 mm.

Fig. 18*a*. Section of foot-gland cells of a normal active animal. Leitz Oc. 12, Obj. 2 mm.

Fig. 18*b*. Section of foot-gland cells of an animal kept for fourteen days in an evacuated desiccator and then placed in water for one and one-fourth hours previous to fixation. Leitz Oc. 12, Obj. 2 mm.

Fig. 19. Section of foot-gland cells from animal dried one week at room temperature. Zeiss Oc. 6, Obj. 2 mm.

Fig. 20. Section of vitellarium from animal dried five days at room temperature, placed in water four hours, then killed and sectioned.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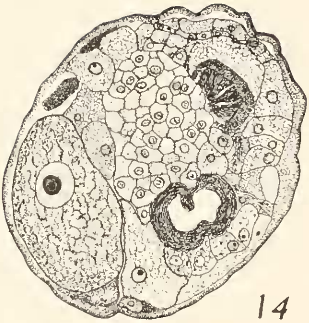
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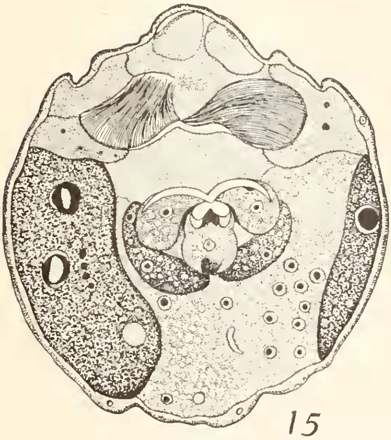
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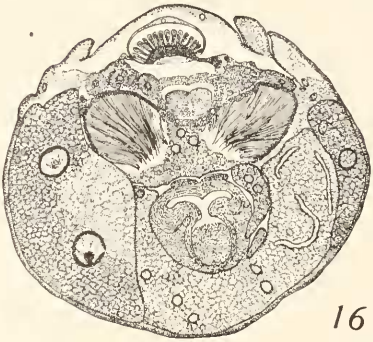
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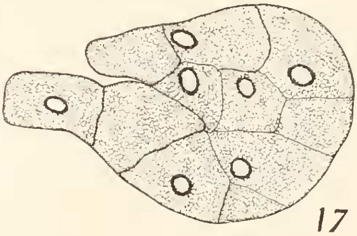
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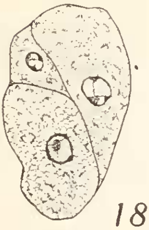
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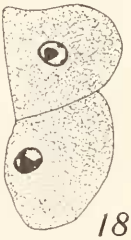
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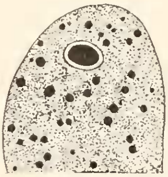
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Plate V.

Fig. 21. Longitudinal section through ovary-vitellarium of a rotifer dried in an evacuated desiccator for fourteen days previous to time of fixation. Leitz Oc. 8, Obj. 2 mm.

Fig. 22. Cross-section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour at the end of which time it was killed. Leitz Oc. 8, Obj. 2 mm.

Fig. 23. Longitudinal section of ovary-vitellarium of animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for one hour and fifteen minutes at the end of which time it was fixed. Leitz Oc. 8, Obj. 2 mm.

Fig. 24. Cross-section of mastax and salivary gland of a normal undried animal. Zeiss Oc. 6, Obj. 2 mm.

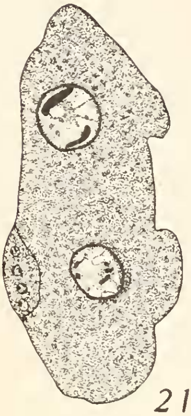
Fig. 25. Section of mastax and salivary gland of an animal dried in an oven at 40° Centigrade for four days. Zeiss Oc. 6, Obj. 2 mm.

Fig. 26. Section of salivary gland cells from an animal desiccated, kept four hours in water, and then killed. Zeiss Oc. 6, Obj. 2 mm.

Fig. 27. Cross-section of brain of *P. roseola*. Normal active animal. Zeiss Oc. 6, Obj. 2 mm.

Fig. 28. Cross-section of brain of a rotifer kept in an evacuated desiccator for fourteen days previous to time of fixation. Leitz Oc. 8, Obj. 2 mm.

Fig. 29. Cross-section of brain of *P. roseola* dried twenty-four hours, put into water for one hour and then killed. Leitz Oc. 8, Obj. 2 mm.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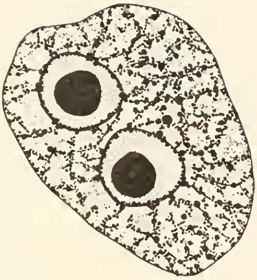
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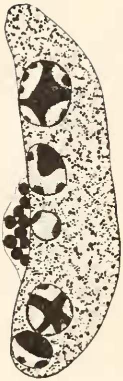
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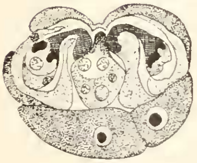
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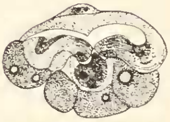
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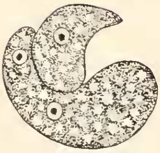
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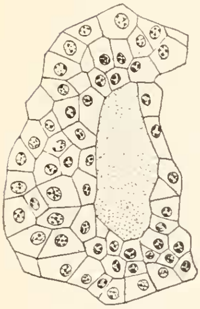
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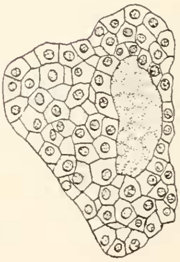
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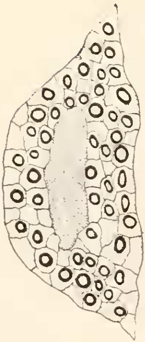
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L. M. HICKERNELL

MITOCHONDRIA AND OTHER CYTOPLASMIC STRUCTURES IN THE SPERMATOGENESIS OF PASSALUS CORNUTUS.

E. L. SHAFFER,

Princeton University, Department of Biology.

A. Introduction.

In course of my study on the spermatogenesis of *Passalus cornutus* (one of the Lacunid beetles), the mitochondria and other cytoplasmic inclusions came to my attention. Although I was primarily interested in the study of the nuclear changes with especial reference to synapsis, it seemed desirable to make a study of the cytoplasmic structures above mentioned, since they showed so clearly in my preparations. I shall therefore reserve for a later report a description of the nuclear changes involved in the course of spermatogenesis.

I am glad to acknowledge my indebtedness to Prof. E. G. Conklin, of Princeton University, under whose guidance and criticism this work has been pursued. I also wish to thank Mr. Milton P. Hunter, of Westtown, Pa., from whom I received the material used in this study.

B. Material and Technique.

There are two pairs of more or less bulb-shaped testes situated in the posterior part of the abdomen. These were dissected out in Ringer's solution and immediately fixed in one of the following fixation fluids: Hermann's, Flemming's strong solution, and Benda's modification of Flemming's fluid. Iron hæmatoxylin followed by either Lichtgrün, erythrosin or Bordeaux red was the stain most used, especially when the fixations were with Hermann's or Flemming's fluids. The Benda crystal violet-alizarin staining method was employed on the material fixed in the Benda fluid. On the whole, material fixed in strong Flemming's fluid (three or four hours) and then stained in iron hæmatoxylin followed by one of the counterstains previously mentioned, was the most satisfactory. In Hermann and Benda material there is a tendency, especially in the spermatocytes, for the mitochondria to agglutinate in dense irregular masses.

C. OBSERVATIONS.

(*a*) *Chromosomes.*

Although not primarily concerned with the chromosomes in the present report, it may be of some interest to at least mention these in a general way. I have as yet been unable to find spermatogonial plates of sufficient clearness to make accurate drawings, but all counts indicate that the chromosome number is 26, including an unequal pair. This has been confirmed by a study of the ovaries in which all dividing follicle cells show 26 chromosomes and these can be arranged in equal pairs (Fig. 3). The metaphase plate of the first spermatocyte (Figs. 12, 33) shows 13 bivalent chromosomes, one of which represents the unequal (sex) pair. The elements of the sex pair separate in the first maturation division and divide equationally in the second division.

(*b*) *Mitochondria.*

I shall not attempt to review the vast literature which has grown up bearing on the subject of the mitochondria. Duesberg ('11) has given a rather complete review of the literature, so that it will not be necessary to do so here. I shall, however, discuss the works of others insofar as these may be related to my observations on *Passalus.*

1. *Spermatogonia.*—In the primary spermatogonia, which are situated at the blind end of the testes, I have been unable to find conclusive evidence of the presence of mitochondria (Fig. 1). The cytoplasm is usually of a homogeneous, non-reticular and finely granular structure. These granules do not stain in anything but the plasma stain, and therefore cannot be considered as mitochondria. There are usually present, in the cytoplasm, several deeply staining bodies (hæmatoxylin) which are perhaps similar to the chromatoid corpuscles of other workers. It is possible that these may really be of a mitochondrial nature, but their subsequent fate cannot in any way be related to the mitochondria of the later stages. Lewis and Robertson (1916) in

their study of the living cells of *Chorthippus* (Orthoptera) found that mitochondria were present in the primary spermatogonia in the form of granular threads; but there is no evidence for such structures in *Passalus*.

The secondary spermatogonia are arranged in cysts in the form of a rosette, and are generally pyramidal in shape. Both nuclear and cytoplasmic volume is noticeably smaller in these than in the primary gonia. The cytoplasm always shows a marked affinity for the hæmatoxylin and this is due to the presence of numerous mitochondrial granules (Figs. 4, 23) which are scattered diffusely throughout the cytoplasm. Payne (1917) found granular mitochondria in the spermatogonia of *Gryllotalpa*, while Duesberg (1910) figures similar structures in *Blaps*. On the contrary Duesberg describes the mitochondria of the gonia of *Blatta* as being present in the form of threads (chondrioconts). Payne, Schäfer ('07) and others have found that the mitochondria of the spermatogonia are localized at the inner ends of the cells (*i. e.*, the end bordering on the cyst cavity). As I have before stated, in *Passalus* the mitochondria are diffusely spread and often are actually absent from the inner ends of the cells. Montgomery (1911) was unable to find evidence of "indubitable mitochondria" in the spermatogonia of *Euschistus*, although he found granules which he considered to be disintegrated idiozome material.

Fig. 5 shows a degenerating spermatogonium. It is noticed that the cytoplasm is much more deeply staining and the mitochondrial granules are much larger. The chromatin of the nucleus is concentrated into one or two karyosomes. The increase in the size of the mitochondrial granules is probably due to an agglutination of the smaller normal ones. Cowdry (1916) in his excellent review on the functional significance of mitochondria, has called attention to the relations of mitochondria in pathological tissues. He mentions the work of Scott who found that in fatty degeneration of the pancreas there was an agglutination of the mitochondria. It is quite possible that the degeneration of cells which is so common in insect spermatogenesis is of the fatty degeneration type. A study of the behavior of the mitochondria in degenerating sperm cells may throw some light on their rôle in normal processes. This is, however, beyond the scope of the present work.

The spermatogonial cysts which are in mitotic activity, stand out very clearly in contrast with the resting cysts. This is because of their lighter staining capacity; whether this is in turn due to the partial disappearance of the mitochondria, could not be ascertained (Figs. 2, 24). Buchner (1909) found that in *Gryllotalpa vulgaris* the mitochondria disappear during or just before cell-division. There are three possible explanations for the partial loss of mitochondrial structure during mitotic activity; (*a*) at this time the cytoplasmic volume is much greater and hence the mitochondria are more diffusely spread; (*b*) the dissolution of the nuclear membrane sets free a large amount of karyolymph which perhaps dilutes the cytoplasm and obscures the mitochondria; (*c*) the mitochondria may dissolve or become chemically changed so that they are no longer recognizable as such. At any rate, they soon appear in great numbers after cell-division, so that their partial disappearance was apparent and not real.

2. *Spermatocytes.*—In the spermatocytes at the beginning of the growth period, the mitochondria are still in the form of diffusely spread granules. There is a noticeable increase in their numbers as the growth period progresses, which plainly indicates that new ones are being formed (Fig. 6). Usually a denser perinuclear zone of cytoplasm can be seen during the growth period; the significance of this zone will be discussed later.

As the late prophase approaches, the cytoplasm becomes filled with numerous delicate threads deeply staining in hæmatoxylin. These are the filar mitochondria (chondrioconts) and they first appear at this time, although there is a slight indication of delicate granular threads in some of the earlier stages. The origin of these threads could not be traced, but it is quite likely that they are genetically related to the granules of the preceding stages. In the first spermatocyte the mitochondria appear diffusely spread when material has been fixed in Flemming's fluid (Figs. 7, 8, 25, 25*a*). In Benda and Hermann material the mitochondria agglomerate in dense irregular masses, still showing their filar nature (Figs. 9, 26). The threads tend to lie with their length extended in the direction of the main axis of the cell. Sections of the cell taken at right angles to its longitudinal axis show the mitochondria on end view (Fig. 25*a*). As the nuclear membrane dissolves and the first maturation spindle is forming (Figs. 10, 29), the mitochondria are usually localized at one side of the spindle. When the spindle has fully formed, the mitochondria begin to envelop it at first from one side (Fig. 11) and later entirely surround the outer spindle fibers, being equally distributed along their lengths (Figs. 13, 30, 31). By the time the metaphase has been reached, the spindle has been entirely surrounded by mitochondria. Figures 12, 32, 33, 34 represent cross-sections at different levels of the first maturation spindle at metaphase, showing clearly the relations of spindle and mitochondria. As is best seen in the anaphase (Figs. 13, 35) they do not entirely reach to the two poles of the spindle, so that there is a short uncovered part near the centrosomes.

The question arises as to the method of movement of the mitochondria in order that they establish this relation with the spindle; is it an active or a passive movement? Many observers on living cells have noted that the mitochondria are generally of a vibratile or motile nature. According to Lewis and Lewis (1914) the mitochondria are never at rest; "a single mitochondrium sometimes twists and turns rapidly as though attached at one end, like the lashing of a flagellum, then suddenly moves off to another position in the cytoplasm as though some tension had been released" (p. 332). If it is true that they possess the power of active movement, it may be possible in this way to explain their movement to the spindle. Lewis and Robertson (1916) state that "the mitochondria migrate from the two masses of mitochondrial granules and elongate towards the poles of the spindle," etc. (p. 108). Whether they mean by this an active or a passive migration is not clear from their account. It seems highly improbable that the movement is active. Some observers have supposed that their movement is influenced by the centrosomes, but there is no conclusive evidence that this is the case. It is more likely that the movement of the mitochondria to their position on the spindle is due to the cytoplasmic movements during metakinesis which Conklin has shown, plays such an important rôle in the localization of cytoplasmic substances in the egg.

There is no evidence from my observations on *Passalus* that the mitochondria divide autonomously, as has been maintained by some workers. As the cell constriction continues through the equator of the spindle (Figs. 14, 35), the mitochondria are divided by this constriction and the daughter cells (second spermatocytes) receive approximately equal amounts of mitochondria. The mitochondrial masses then move a short distance toward the poles; this is evidently caused by a further elongation of the spindle. Montgomery (1911) has described a similar method of division in *Euschistus*. The fact that mitochondria are found in equal amounts in daughter cells is supposed by some to favor the view that they divide autonomously; but it is well known that yolk and similar substances are often found in equal amounts in daughter cells, yet no one maintains that yolk granules divide autonomously. Fauré-Fremiet (1911) describes autonomous division in the mitochondria of the Protozoa, and Wilke (1912) does likewise for the spermatocytes of *Hydrometra*. Payne (1917) does not incline strongly to the view that the mitochondria are divided by the cell constriction, while Lewis and Robertson (1916) do not clearly state whether their division in *Chorthippus* is autonomous or passive.

Following the first maturation division, there is a short interkinesis during which the second maturation spindle is formed (Figs. 16, 36). At this time the mitochondria are lying at one side in a rather dense mass which shows a lighter central portion through which the spindle of the previous division passed. The behavior of the mitochondria during the second maturation division is precisely the same as in the first division (Figs. 17, 37). They again surround the spindle peripherally and are divided by the equatorial constriction. As a result of this division both spermatids receive approximately equal amounts of mitochondria (Fig. 18).

The mitochondrial mass contained in the spermatid gradually becomes more compact and henceforth may be designated as the Nebenkern. It stains intensely with the basic stains and often shows a granular structure. It often shows a lighter central portion which represents the position the spindle had occupied (Fig. 20*d*). Soon after the Nebenkern has become a spherical body, it begins to show a peripheral lighter area which is of a vacuolar nature (Fig. 19). As the spermatid elongates the Nebenkern becomes elongated and the vacuolization of the peripheral layer becomes more marked (Fig. 20, *a*, *b*). It becomes divided into two halves and is later pierced by the axial filament (Figs. 21, 39). As the axial filament grows out, the Nebenkern continues to elongate forming a sheath about it. The material of the Nebenkern thus extends from the centrosome for a considerable distance along the axial filament (Fig. 22). Fig. 20, *c* is that of a cross-section through the tail of a spermatid showing the two halves of the Nebenkern on each side of the axial filament. Whether or not the Nebenkern sheath extends to the free end of the axial filament could not be determined. As the transformation continues, the Nebenkern becomes more lightly staining, and all evidences of its mitochondrial nature are lost.

3. *Discussion.*—I have been unable to obtain any definite and conclusive evidence as to the manner in which the mitochondria arise. There can be no doubt that they increase in numbers during the growth period, and the question arises: to what influence do the mitochondria owe their origin? This has been and still is a much-debated cytological question, and much depends on the final solution of the problem.

Meves, Bouin, Duesberg and others have always maintained that the mitochondria are persistent and self-perpetuating structures, much as the chromosomes of the nucleus are at present regarded. On the other hand, Goldschmidt and his pupils, Buchner, Jordan, Wildman and others have derived the mitochondria from the nucleus, and have thus maintained that they are akin to the chromidia of Hertwig. Vejdovský has traced their origin from the sphere material, while Montgomery in *Euschistus* concludes that they are probably derived from the idiozome or the nucleus, or by a "joint action" of both. In their studies on the living sperm cells of *Chorthippus*, Lewis and Robertson are unable to find any evidence that the mitochondria are derived from the nucleus. It is thus evident that the facts are conflicting on all sides. If it is true, as I have previously stated (p. 408), that the mitochondria are not present in the primary spermatogonia of *Passalus*, then we cannot maintain that they are persistent and self-perpetuating. I am, however, not entirely convinced that they are absent in the primary spermatogonia and shall await fresh material for a study of the living cells.

It is possible that the increase in number of the mitochondria during the growth period is due to a simple growth and division of those already present. I cannot find any evidence for this in my material. In nearly all the growth stages of the first spermatocytes, there is present a denser and more deeply staining perinuclear zone. Schäfer (1907) has figured similar conditions in *Dytiscus*, while Voivnov (1903) shows it more strikingly in *Cybister* and calls it the "zona interna." During the maturation divisions, the "zona interna" surrounds the spindle peripherally and forms the Nebenkern of the spermatid. As Duesberg (1910) has pointed out, it is quite evident from this behavior that the "zona interna" is really of a mitochondrial nature. Giardina (1904) has studied the oöcytes of a number of forms (*Periplaneta*, *Stenobothrus*, *Gryllus*, *Mantis*, etc.) and figures well-defined perinuclear zones usually of a granular nature. Whether this zone is formed by a diffusion of chromatin in the form of a solution out of the nucleus into the surrounding cytoplasm or whether it arises in situ in the cytoplasm by interaction with the nucleus, is a question which Giardina discusses at considerable length. He concludes in favor of the latter view. Payne (1917) has described a similar perinuclear zone in the oöcytes of *Gryllotalpa*, but he definitely states that this consists of mitochondria. As the oöcyte grows, the mitochondria migrate centrifugally into the cytoplasm. Vejdovský (1911–12) shows the mitochondria arranged in a definite perinuclear zone in the spermatocytes of *Diestramena*. It thus seems quite probable that the perinuclear zones described by Giardina are really of a mitochondrial nature. The occurrence of this zone in such close connection with the nucleus and especially during a period when the mitochondria are certainly increasing in number, is highly indicative of an interaction between nucleus and cytoplasm. The relation of this zone to the mitochondria seems to be a strong argument, at least, that it is the locus of mitochondria formation.[1]

The association of the spindle and the mitochondria has led to some confusion in regard to the origin of the Nebenkern, so that some workers have ascribed its origin to the spindle remains of the second maturation division. Arnold (1908) comes to this conclusion in his study of *Hydrophilus*, as does Baumgartner in *Gryllus*. Voivnov, as previously mentioned, derived the Nebenkern from his "zona interna" and the peripheral spindle fibers. Munson (1906) in his work on *Papilio* also derives the Nebenkern from the spindle remains, but his observations are interesting since they clearly show that he has confused these with the mitochondria. According to his view, it is only the "outer, granular" mantle fibers of the spindle which take part in the formation of the Nebenkern. It is evident, in light of the recent work, that the "outer, granular" mantle fibers are really mitochondria. That he actually saw the mitochondria and misinterpreted them, is evident from the following quotation: "Often a few scattered chromatin segments are found scattered along the spindle fibers, or else drawn out into stainable threads parallel with the spindle fibers" (p. 91).

(*c*) *Spindle Derivatives.*

When one attempts to review the literature on the subject of spindle derivatives and their histories, one is immediately confronted with a maze of conflicting observations and interpretations, to say nothing of a nomenclature which is almost hopelessly confused. The names mitosome, idiozome, attraction-sphere, centrosphere, astrosphere, Nebenkern (of older workers), etc., are all examples of the existing confusion, and should caution us against hasty interpretations of such structures. Meves (1899) states that he first applied the term "idiozome" to those compact bodies in the spermatogonia and spermatocytes which surround the central corpuscles (centrosomes). By a disintegration of the idiozome, the centrosomes are set free and then take part in the next cell-division. In the rat, however, Meves found that there is no disintegration of the idiozome, but that the centrosomes wander out, leaving the idiozome intact. As division progresses, the latter dissolves and disappears. There is no essential difference between the "attraction-sphere" of Van Beneden, the "centrosphere" of Strasburger and the "astrosphere" of Fol and Boveri; and so far as I have been able to ascertain, there is no fundamental difference between these last named structures and the "idiozome" of Meves. One thing is clear,—that these structures all refer to the achromatic substance of the spindle situated at the poles and usually enclosing the central corpuscles. To avoid any possible misuse of these terms I shall employ the non-committal term "sphere" to denote this portion of the spindle. With regard to the remains of the spindle proper, there is less confusion of terms, and I shall use the term "mitosome" or "spindle remains" to designate this structure.

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[1] It is interesting to note in this connection that the material of the yellow crescent in the ascidian egg has been shown by Conklin to be found at various times in a perinuclear position; and Duesberg (1913) has shown that this zone is extremely rich in mitochondria.

1. *Spermatogonia.*—The spermatogonia in *Passalus* in mitotic activity stand out very sharply in contrast with those in the resting condition not only because of their lighter staining capacity (as mentioned on p. 410), but also because they tend to become round in outline and the cell walls become more sharply defined. This is indicative of an internal pressure which Reinke (1900) calls the "mitotic pressure." As the gonial anaphase progresses, a well-defined cell-plate makes its appearance and stains deeply in hæmatoxylin (Figs. 2, 24). As the telophase advances, the spindle and cell-plate become more compact, the former taking the acid stains and the latter taking the basic stains. Fig. 4 shows several resting cells from a spermatogonial cyst with the spindle remains (mitosome) running from cell to cell. Very often the spindle remains from each division become so connected that they form sort of bond between all the cells in a cyst. Hegner (1914) has described a similar condition in the spermatogonial cysts of *Leptinotarsa decemlineata.* In this case, however, he found that material fixed in Carnoy's fluid always showed the spindle remains taking the basic stain.

Günthert (1910) has described similar results in the differentiation of nurse cells and oöcytes in *Dytiscus* and Govaerts (1913) has done likewise in *Carabus*. In *Passalus*, it is only in the region of the cell-plate that the spindle takes the basic stain. In cross-section the spindle remains appear as plasmasome-like bodies in the cytoplasm. Often there may be seen deeply staining granules adhering to the surface of such bodies; these I take to be portions of the deeply-staining cell-plate.

2. *Spermatocytes.*—The spindle remains of the last spermatogonial division (together with the cell-plate) persist into the spermatocyte and remain very conspicuous throughout the entire growth period (Figs. 6, 8, 27). Every spermatocyte exhibits these spindle remains, either in their original positions connecting cells or else in the form of plasmasome-like bodies lying free in the cytoplasm. Similar conditions have been shown by Voivnov in *Cybister* and by Munson in *Papilio*. In *Gryllotalpa*, Payne states that "there is no indication that a sphere or the spindle and astral fibers persist after cell-division." In his figure B, plate 1, he shows two plasmasome-like bodies present in the cytoplasm of a spermatocyte during the early growth period. As to the origin of these bodies, he is uncertain, but he is not ready to admit that they may be idiozome material. I agree that it would be rather speculative to assign the term "idiozome" to these bodies, but in *Passalus* it is quite clear that similar bodies are derived from the spindle. I am not able to establish the presence of a definite "idiozome" or sphere material in the spermatocytes of *Passalus* and just where the centrosomes lie hidden during the growth period is a difficult matter to determine. Since the cells are so filled with mitochondria at this time, it seems almost impossible to definitely locate them. The centrosomes are first discernible when the first maturation spindle makes its appearance. From the long persistence of the spindle remains of the last spermatogonial division, it may be concluded that its substance must be of some inert, resistant material. Munson (1906, p. 90) makes the following remarks on *Papilio:*

"The resistance of the maturation spindles to reagents is remarkable. In studying the living dividing cells on the slide, I have seen the cytoplasm gradually disintegrate, become vacuolated, and disappear while the spindles remain as perfect as ever. In testing the effects of reagents, too, I have been able to dissolve practically the whole of the cytoplasm of all the cells of the cyst, while the spindles remained, showing a system of connected spindles throughout the whole cyst."

There are other structures related to the spermatocytes which are of a more or less problematical nature. In Figs. 8 and 28 there will be noticed a large vacuole in each of the spermatocytes; these are to be found in nearly every spermatocyte during the late prophases and thus far I have been unable to find them in the earlier stages. The vacuoles are filled with a homogeneous fluid (perhaps a gel) which takes the plasma stain but lightly. Sometimes there may be several vacuoles in a single cell. In cysts of spermatocytes having such vacuoles, one often finds in the cyst cavities round or oval bodies of a granular nature (Fig. 8) resembling very much the cytoplasm of the spermatocytes. Furthermore, in such cysts the sides of the spermatocytes bordering on the cyst cavity are of an irregular outline and often distinct pseudopod-like projections of the cytoplasm are given off. It is therefore highly probable that the small bodies found free in the cyst cavity have arisen from the pseudopod-like projections or buddings from the cytoplasm of the spermatocytes. What the significance of either the vacuoles mentioned above, or of the casting off of portions of the cytoplasm may be, I am unprepared to state. The presence of vacuoles and pseudopods at the same time may indicate that the vacuoles are cast out by means of setting free the pseudopods. But this view is at present untenable, for I have never actually seen vacuoles within the pseudopods; furthermore the contents of the vacuoles are always of a homogeneous appearance, while the cast off parts of the cytoplasm are granular as described above. Voivnov has found in *Cybister* that vacuoles make their appearance in the late prophases of the spermatocytes. These differ in appearances, at least, from those found in *Passalus* in that they contain definite bodies as inclusions. Voivnov also finds that they are later cast out into the cyst cavity. He considers these to be portions of the sphere (idiozome) which are undergoing disintegration. In support of this view, he finds that they appear at a time when the centrosomes are first set free and often they may be found in close spatial relations to the centrosomes.

Whether or not the vacuoles and the cast-off portions of the cytoplasm in *Passalus* correspond to the conditions described by Voivnov in *Cybister* I am at present unable to say. The origin of the vacuoles and their relation to the cytoplasmic buddings can be best studied in the living material, and I shall leave the question undecided here for lack of evidence.

3. *Maturation Divisions and the Spermatid.*—The spindles developed for both maturation divisions are relatively very large, both in actual dimensions and in number of spindle fibers. Correlated with the large size of the spindles is the relatively large nucleus, containing little chromatin but a large amount of karyolymph. The view of Conklin and others, that the spindle grows at the expense of the karyolymph is certainly substantiated in the case of *Passalus*. In both maturation divisions cell-plates are developed which are very much smaller than those found in the spermatogonia (Figs. 14, 8), and the remains of the spindles persist for a considerable time after division. In the anaphase of the second maturation division, the centrosomes are still distinguishable lying close to the chromatin masses at the poles. In the telophase of this division they are found lying on the nuclear membrane. It is because of this position on the nuclear membrane that they are not easily detected, but careful searching and focusing will show their undoubted presence (Fig. 18). Thus in the spermatid, the centrosome is still to be seen closely attached to the nuclear membrane; it gradually shifts its position until it comes to lie between the nucleus and the Nebenkern. Usually there is a precocious growth of the axial filament (Fig. 19) before the centrosome arrives at its ultimate position. At this time, the centrosome appears double, while the axial filament grows out between the two halves of the Nebenkern and becomes associated with the latter in the formation of the tail, as shown before (p. 413). As transformation continues (Figs. 21, 22), the centrosomes become more and more closely associated with the nucleus, until in the older stages they are scarcely

distinguishable from the head of the spermatozoön, and hence there is no well-defined middle-piece.

In the youngest spermatid there is always present a cytoplasmic body of a refringent nature which takes the plasma stain (Figs. 19, 38). One portion of it is more compact, while the other part is in the form of a vacuole containing a deeply staining (hæmatoxylin) body. This structure is undoubtedly a spindle derivative, but whether it represents the sphere material or the mitosome I am unable to state. As to the origin of the deeply staining body contained within it, I am equally unable to explain. It may possibly represent a portion of the cell-plate which has become detached from the cell wall and has become encompassed in the remains of the spindle.[1] In older spermatids a compact portion of the spindle derivative is found lying near the nucleus in that part of the spermatid which is destined to give rise to the head end of the spermatozoön (Fig. 21). In later stages, as the nucleus becomes laterally compressed, this structure is transformed into the acrosome. The remainder of the spindle derivative is sloughed off into the tail of the spermatozoön and gradually disappears.

4. *Discussion.*—As has been previously stated, the axial filament in *Passalus* arises in connection with the centrosome. Munson (1906) has expressed an entirely different view concerning its origin in *Papilio.* His view is that the axial filament represents a much compressed portion of the cytoreticulum and has no relation to the centrosome. Accordingly, he finds that in the stages of development of the axial filament occasionally three or four filaments may be present in a single spermatid, but these finally unite into a single thread. Paulmier (1899) has described double and quadruple spermatids in which two or four axial filaments were present, but each was connected to a centrosome. Munson's view arises from the fact that he has assigned a wholly different function to the centrosome. His figures of the spermatids of *Papilio* show spindle derivatives each containing a deeply staining body which he interprets as

[1] Duesberg (1908) figures a similar spindle derivative in the spermatid of the rat which he designates as the "idiozome." It also consists of a vacuolar portion which contains a deeply-staining granule which is not a centrosome, just as in *Passalus.*

the centrosome. The conditions here are very much like those in *Passalus* (Figs. 19, 38), but in the latter case the deeply staining body is certainly not a centrosome. The sphere (?) of the spermatid of *Passalus* has no connection with the centrosome. This is in agreement with Montgomery's conclusions in *Euschistus*. According to Munson, the centrosome gives rise to the acrosome of the spermatozoön.

Payne (1917) has approached the subject of spermatid transformation with more or less scepticism as to the generally accepted origins of the structures present here. I am in hearty accord with this point of view, but it seems that Payne has carried matters too far. I agree that to call the structure from which the axial filament grows a centrosome without tracing its history from the second spermatocyte, is highly speculative. But to say, as Payne does, that at a certain stage of the spermatid "there is nothing in the cytoplasm but mitochondria" (p. 309) is equally as dogmatic and unwarranted in light of the observations of many other workers. In *Passalus* there can be no doubt that the centrosome of the spermatid has actually been carried over from the preceding cell-division. Furthermore, in the youngest spermatid there can always be found the refringent cytoplasmic body which is undoubtedly a spindle derivative. In *Gryllotalpa*, Payne finds in the stages succeeding the young spermatids which contain no other cytoplasmic structures but mitochondria, the sudden appearance of two deeply staining bodies, one of which later forms the acrosome, while the other is pushed off into the tail. Since these were not present in the earlier stages, they have apparently arisen "de novo," that is, they are newly differentiated parts of the cytoplasm. One might then expect to find developmental stages of such structures, but Payne does not indicate such. However, it seems to me that the building-up of the spermatozoön from the spermatid is a process involving no differentiation, but a *transformation* of differentiations already present. All the structures needed in the building up of the spermatozoön are at hand, and there is no further elaboration of new ones. Munson (1906, page 96) has clearly expressed a similar view:

"The comparative inertness of the nucleus at the close of the last maturation division and ever afterward would not justify us in assuming that this is a growth period in the history of the spermatozoön; but rather that it is a transformation period of those organs that are already present and fully-grown in the spermatid stage. This transformation in all the organs of the cell is merely an elongation such as could be brought about, doubtless, by prolonged lateral pressure."

D. General Considerations.

The importance of the mitochondria as bearers of hereditary units rests on their mode of origin and maintenance through the cell cycle, their behavior in fertilization and their rôle in differentiation. If, as has been previously discussed, the view is correct that the mitochondria owe their origin to materials derived from the nucleus or by the activity of the nucleus, then their importance in heredity can only be secondary.

Just how much of the spermatozoön enters the egg is a matter of importance in ascertaining the rôle of the mitochondria. In *Nereis*, Lillie (1912) has found that the middle piece and the tail of the spermatozoön do not enter the egg. On the other hand, Meves (1911) has shown that the entire spermatozoön enters the egg of *Ascaris*, and Van der Stricht (1909) has shown similar results in the bat. It therefore seems impossible to make any generalizations on this subject until more work on the details of fertilization has been done. In the case of *Peripatus*, Montgomery (1912) has shown that the mitochondria are entirely lost in the spermatozoön, being thrown off within certain cytoplasmic lobes. Here, at least, the mitochondria of the spermatozoön can play no part in the transmission of hereditary characters.

The mitochondria have been most exhaustively studied in somatic cells where they are present in a variety of forms. Certain workers (Meves, Duesberg, Hoven and others) have maintained that the mitochondria give rise to myofibrils, neurofibrils and other somatic differentiations; but these views have not been strongly substantiated. The work of Cowdry (1914) is strong evidence that the mitochondria of nerve cells are not transformed into neurofibrils.

The researches on the chemistry of mitochondria are practically all in agreement that they are phospholipins or lecithin-albumins. No one has attempted to show that yolk is a bearer of hereditary units; yet yolk is chemically allied to mitochondria. In fact Fauré-Fremiet has shown that mitochondria actually transform into yolk. The work on the chemistry of mitochondria indicates that they are of great importance in the metabolic activity of cells, but our knowledge of their relation to heredity is negative. To say that the mitochondria are not the bearers of hereditary units is not denying that they may *influence* heredity in some cases, just as we know that heredity can sometimes be influenced by environmental conditions of food, temperature, etc.

One fact which has come clearly to light from this study is that beginning with the spermatogonia and continuing up to the spermatid, there is a progressive elaboration of mitochondria. They are then transformed into a definite structural part of the spermatozoön, the sheath of the axial filament. This progressive increase in the amount of mitochondria seems to indicate that they are differentiation products. Hence if there is any genetic continuity between the mitochondria of successive cell generations, it is only of a limited sort. The conception that the mitochondria present in the somatic cells are the direct descendants of those of the germ cells from which they have arisen, certainly has very little evidence in its favor. It seems more probable that mitochondria are in the nature of cytoplasmic differentiations, akin to metaplasm (yolk, etc.) and without a definite relation to the development of hereditary characters, but with the capabilities of influencing development insofar as they may be related to the metabolic activity of cells. It is possible that in the spermatozoa, the mitochondria merely function as locomotory organs.

SUMMARY.

1. Although mitochondria can not be definitely demonstrated in the primary spermatogonia of *Passalus cornutus*, they are present in the secondary spermatogonia in the form of numerous and diffusely spread granules.

2. The mitochondria increase in number during the growth

period, and in the later stages are found in the form of threads (chondrioconts) which lie in the direction of the chief axis of the cell.

3. During the maturation divisions, the mitochondria envelop the spindle peripherally and are divided by the cell constrictions, so that daughter cells receive approximately equal amounts.

4. The mitochondria of the spermatid form the Nebenkern, which later is pierced by the axial filament. As the latter grows, the Nebenkern elongates, forming a sheath about it.

5. Spindle remains are found forming connections between the spermatogonia. The spindle remains of the last spermatogonial division persist throughout the entire growth period of the spermatocyte.

6. A spindle derivative is found in the spermatid, a portion of which gives rise to the acrosome of the spermatozoön.

7. The centrosome of the second maturation division is carried into the spermatid and gives rise to the axial filament of the spermatozoön. The centrosome becomes so closely associated with the nucleus that there is no well-defined middle-piece in the spermatozoön.

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ABBREVIATIONS.

c., centrosome; *m.*, mitochondria; *N.*, Nebenkern; *s.*, spindle derivative.

All drawings were made with the aid of a camera lucida at table level using a Zeiss 12 ocular and a 2 mm. oil objective. The reproductions have been reduced one-third.

EXPLANATION OF PLATE I.

Fig. 1. Primary spermatogonium. No clear indication of the presence of mitochondria. Note small mass of chromatoid substance in cytoplasm.

Fig. 2. Anaphase of secondary spermatogonium. Note lightly-staining cytoplasm, well-defined cell-plate, and the tendency of the cell to become round.

Fig. 3. Metaphase plate of ovarian follicle cell, showing thirteen equal pairs of chromosomes.

Fig. 4. Resting stages of secondary spermatogonia. Mitochondria granular and diffusely spread. Note persistence of spindle and cell-plate.

Fig. 5. Degenerating spermatogonium. Mitochondria larger in size; chromatin concentrated in two large karyosomes.

Fig. 6. Pachytene stage of first spermatocyte. Mitochondria still granular, with only a slight indication of threads forming; note denser perinuclear zone and the persistence of the spindle remains of the last spermatogonial division.

Fig. 7. Late prophase of first spermatocyte, Flemming fixation. Nucleus uncut. Mitochondria filar, with their lengths in the direction of the chief cell axis.

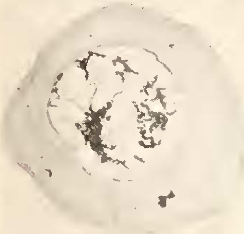
Fig. 8. Similar stage as above, showing remains of spindle still persisting, two large cytoplasmic vacuoles of problematic origin, and small bodies in the cyst cavity which have been budded off from the cytoplasm of the spermatocytes.

Fig. 9. Prophase of first spermatocyte, Hermann fixation; showing agglomeration of the mitochondria.

Fig. 10. Formation of the first maturation spindle; mitochondria at one side.

Fig. 11. Mitochondria beginning to envelop first maturation spindle.

Fig. 12. Cross-section of the metaphase plate of first spermatocyte, showing mitochondria completely surrounding the spindle. Thirteen bivalent chromosomes.



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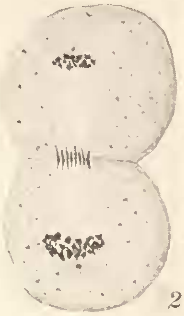

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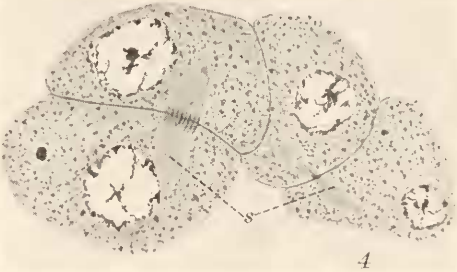

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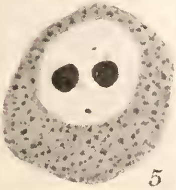

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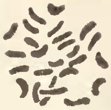

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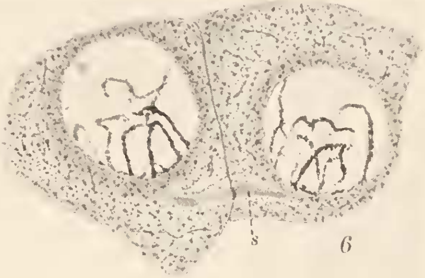

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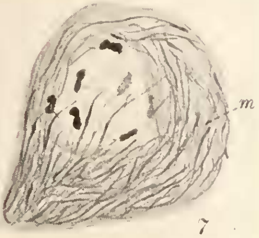

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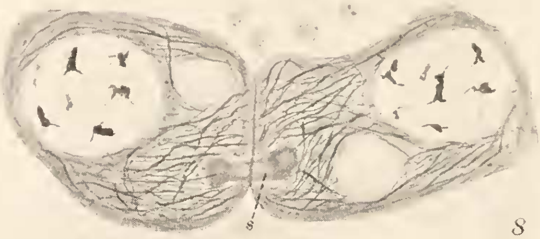

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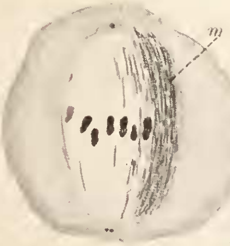

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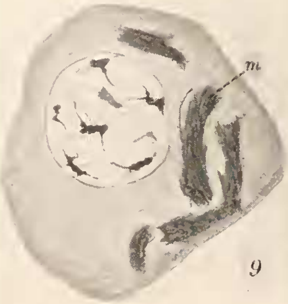

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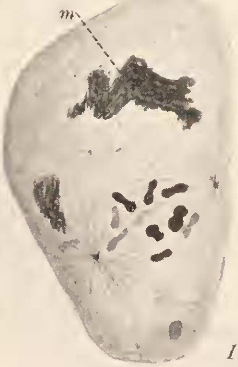

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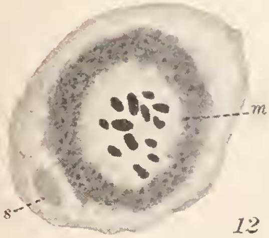

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EXPLANATION OF PLATE II.

Fig. 13. Anaphase of first spermatocyte, showing relation of spindle and mitochondria.

Fig. 14. Late anaphase of first spermatocyte. Cell constriction has divided mitochondria so that the daughter cells contain approximately equal amounts.

Fig. 15. Cross-section of first spermatocyte near pole of spindle, showing absence of mitochondria here.

Fig. 16. Second spermatocytes (interkinesis). Mitochondria present in daughter cells in compact masses, also showing the persistence of the spindle and cell-plate.

Fig. 17. Metaphase of second spermatocyte. Mitochondria again surrounding the spindle peripherally.

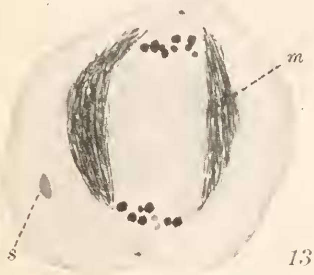
Fig. 18. Telophase of second maturation. Mitochondria again divided by the cell constriction into two equal masses. Note position of the centrosome (*c*) lying on the nuclear membrane.

Fig. 19. Spermatid. Nebenkern (*N*) derived from the mitochondria, with a peripheral vacuolated portion. Persistence of centrosome (*c*) with a precocious growth of the axial filament; spindle derivative (*s*) enclosing a deeply-staining body.

Fig. 20. Changes in the Nebenkern during transformation of spermatid. *c*, cross-section of the tail showing the Nebenkern lying on each side of the axial filament. *d*, Nebenkern with lighter central portion where spindle of previous division had passed through.

Fig. 21. Stage in the transformation of the spermatid. A portion of the spindle derivative (*s*) occupies a position at the head end and a portion passes into the tail. Nebenkern elongating, and the axial filament growing out of the centrosome (*c*) between the two halves of the Nebenkern.

Fig. 22. Later stages in the transformation of the spermatid. *b*, giant spermatid.



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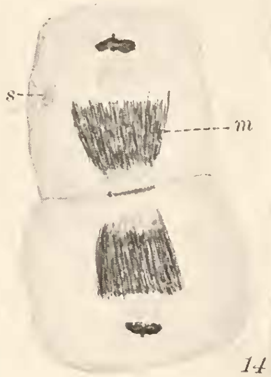

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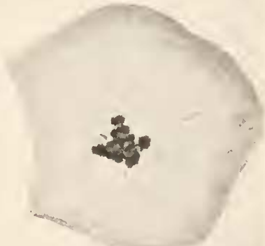

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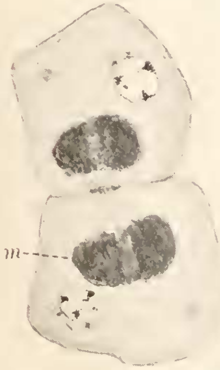

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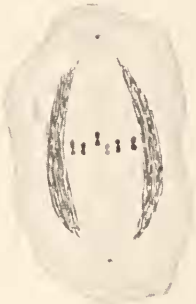

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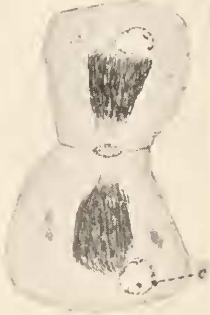

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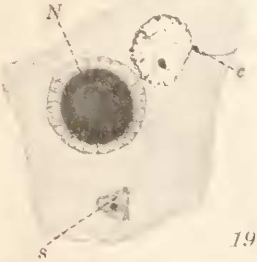

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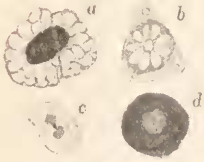

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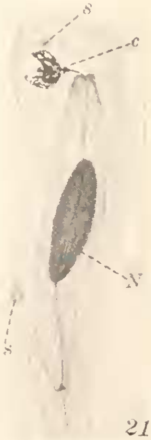

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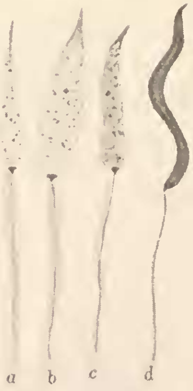

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EXPLANATION OF PLATE III.

Photomicrographs taken at a magnification of about 1,500 diameters

FIG. 23. Resting secondary spermatogonia, showing diffuse granular mitochondria deeply staining in iron-hæmatoxylin.

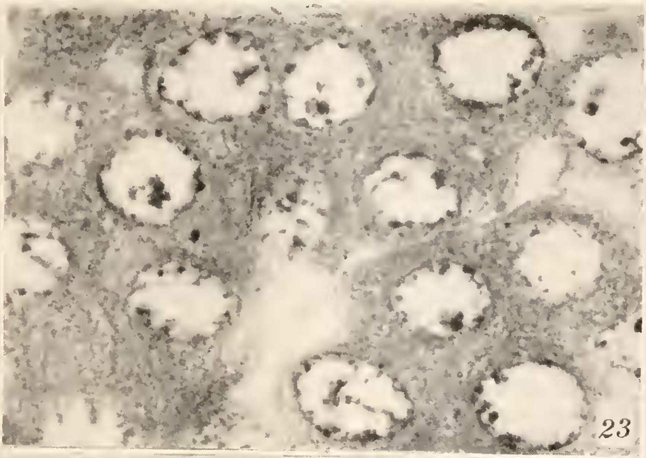
FIG. 24. Telophase of a secondary spermatogonium, showing the deeply staining cell-plate. The rest of the spindle here shows somewhat darker than it really is; it always takes the plasma stain.

FIG. 25. Late prophases of first spermatocyte; Flemming fixation. The mitochondria are filar, with their lengths extended in the direction of the chief axis of the cell; the nuclei here are uncut and somewhat out of focus. Fig. 25*a* is a section at right angles to the chief axis of the cell and shows the mitochondria on end view.

FIG. 26. First spermatocyte from a Hermann fixation. The mitochondria are agglomerated in dense masses (*m*).

FIG. 27. Part of a cyst of first spermatocytes to show the spindle remains (*s*) forming a connection between several cells; parts of it stain deeply with iron-hæmatoxylin.

FIG. 28. First spermatocyte greatly destained to show the cytoplasmic vacuole of unknown significance.



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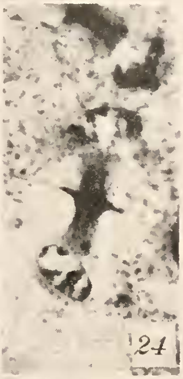

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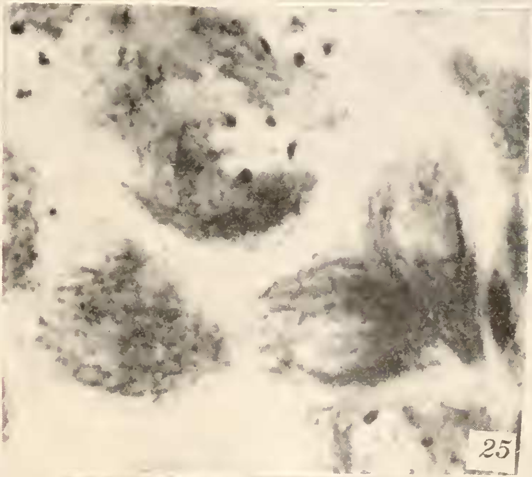

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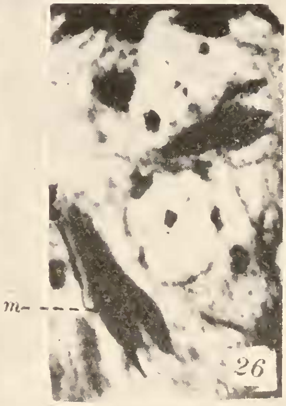

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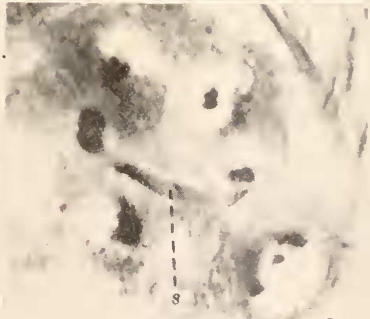

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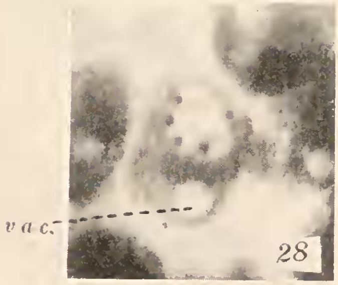

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EXPLANATION OF PLATE IV.

FIG. 29. Formation of first maturation spindle; the mitochondria are at one side and are beginning to envelop the spindle.

FIG. 30. Metaphases of first maturation division; the mitochondria completely surround the spindle.

FIGS. 32, 33, 34. Cross-sections of the first maturation spindle, showing its relation to the mitochondria.

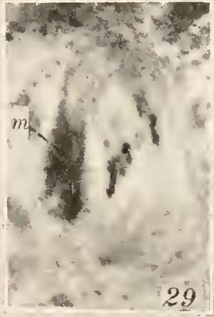
FIG. 35. Late anaphases of the first maturation division; the mitochondria are densely staining and closely applied to the spindle. The mitochondria do not reach to the poles.

FIG. 36. Second spermatocyte; interkinesis. Mitochondria in a dense mass awaiting the formation of the second maturation spindle.

FIG. 37. Second maturation spindle forming with the mitochondria at one side.

FIG. 38. Young spermatid with deeply staining Nebenkern (*N*) lying close to the nucleus, and the spindle derivative (*s*) which contains a deeply staining corpuscle. Centrosome not clearly in focus.

FIG. 39. Later stage in spermatid transformation, showing the elongation of the Nebenkern after the axial filament has grown out.



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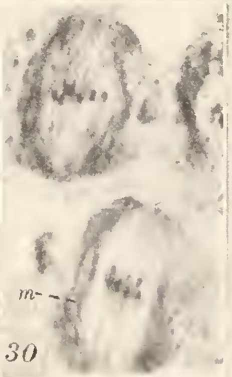

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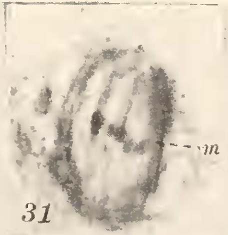

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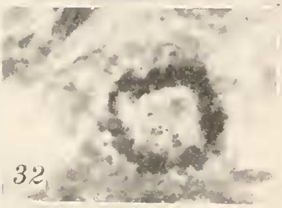

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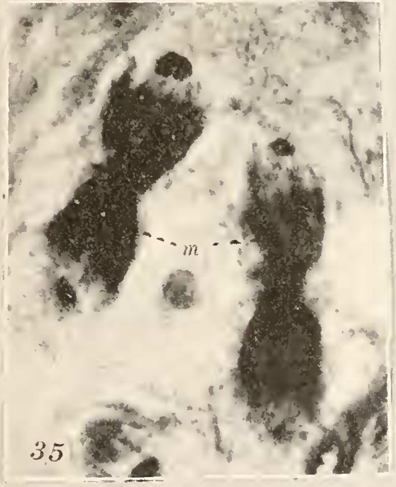

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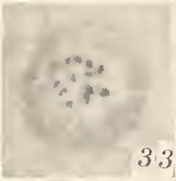

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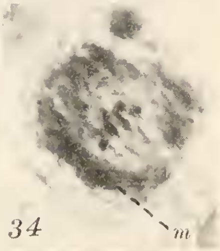

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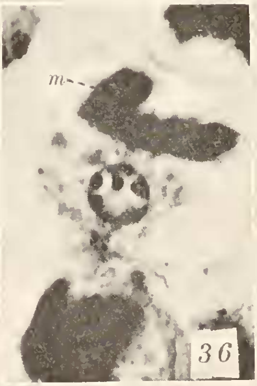

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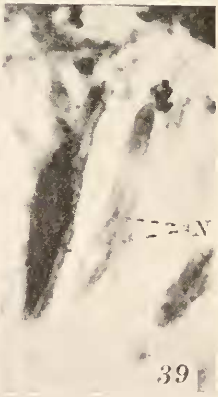

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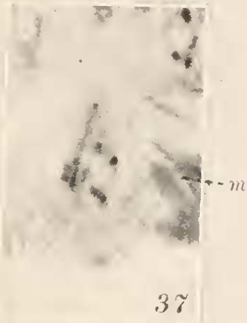

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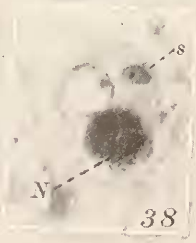

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